JOLINE MUNOZ
GRANT LANDRUM

Executive Summary

The alternative fuel biodiesel can be used in current diesel engines just like regular petroleum diesel. Biojewicz Biodiesel has explored the guidelines for a biodiesel production facility that uses algae as an oil source. Algae uses the least amount of land in comparison to other oil producing crops. The production facility is to be located next to a natural gas power plant for its flue gas supply of carbon dioxide, its location in a high concentrated sunlight area and its abundant brackish water source of an underground aquifer. Several different scenarios were evaluated for this facility; however, two different options were focused on in detail. The larger the lipid content of the algae yields greater production rates of biodiesel. The lipid content of the algae can be manipulated by algae starvation or via genetic engineering. In addition to biodiesel, glycerol product is produced and sold raw, and the remaining biomass (sugars) after the oil is extracted are fermented to produce xanthan gum, a valuable commodity that is used in multiple industries. Several different possibilites for byproducts are discussed, but the fermentation of sugars to form xanthan gum proves to be the most profitable. The options considered the varying algae lipid content of 20, 30, and 40% and the various land usage of 1, 4 and 7 square miles. The only profitable option at $0.72/gallon of biodiesel is a 7 square mile algae production with an algae lipid content of 20%. The NPW is $12.6 million with a return on investment (ROI) of 3%. Risk was assessed and the lower lipid content showed in both of the main evaluated options that were the main focus to be the most promising to yield a profit at a biodiesel selling price of $0.72/gallon. This is due to the fact that at this selling price, the byproduct fermentation is more profitable than the biodiesel production. The biodiesel production profitability surpasses that of the fermentation at a selling price of $3.14/gallon. With this higher selling price, as would be expected, there is a greater NPW of $203 million with a ROI of 56%.

Table of Contents

Table of Tables

Introduction

Biojewicz Biodiesel is an environmentally friendly company that promotes the growth of green energy through the alternative fuel, biodiesel. The company is lead by two chemical engineers from the University of Oklahoma. The company plans to implement a biodiesel production plant with a main oil source of algae. The facility will be located near a power plant that exhausts excessive amounts of carbon dioxide daily. The company wants to lead by example and encourage other companies to venture out into the newer alternative fuel technology.

Objectives

With this first facility, the engineers will use their expertise to optimize the entire process and find ways to make the technology easier to implement. The company will emphasize the importance of reducing #1 the United States great dependence on foreign oil and #2 green house gas emissions. Biojewicz Biodiesel wants to take the lead in the biodiesel market before the boom.

Future

Future plans are to do further research on different locations and the specific algae specimens that are abundant in those environments. Further research will also be aimed toward the genetic engineering of these algae species to produce more lipids and towards alternative ways to harvest different size algae.

Introduction

The United States consumes 60 billion gallons of diesel (**Widescale Biodiesel Production from Algae**) each year, with ~~much~~ most of its oil being supplied by the rest of the world. (US Consumption, Production). The bulk of the diesel is consumed by the transportation sector. Texas alone consumes 8 percent of the diesel used nationally on highways and roads. In addition, Texas also accounts for 11 percent of the U.S. consumption of diesel by off road vehicles and equipment (Source from online?). Currently, research has been invested into alternative energy sources to reduce dependence on petroleum. One of those sources, biodiesel, has environmentally friendly qualities that will reduce green house gas emissions and help feed the increasing demand for diesel. This will reduce the current strain on diesel refineries to meet such high demand, while simultaneously stimulating the country's economy by increasing jobs. Another good quality of biodiesel is that it can be used as a supplement in # 2 diesel fuels by a process as simple as adding a portion of biodiesel to the current petroleum diesel in blends of 5, 10, or 20 percent, with the most common blend being B20, or 20 percent biodiesel. The blended diesel may then be used in standard diesel engines with the only side effect being that it cleans your engine as it is burned.

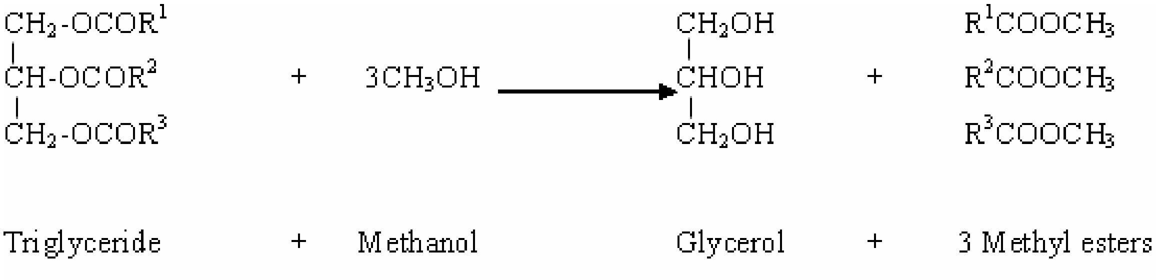
Company Objectives

Biojewicz Biodiesel is an environmentally friendly company that promotes the growth of green energy through the alternative fuel, biodiesel. The company, lead by two chemical engineers from the University of Oklahoma, plans to implement a large scale biodiesel production facility. In doing so, Biojewicz Biodiesel hopes to encourage other companies to venture into the innovative alternative fuel.

With its first facility, the engineers at Biojewicz Biodiesel will use their expertise to optimize the entire process, from initial oil source selection to byproduct usage. In addition, Biojewicz Biodiesel hopes to ease the implementation of this new technology for future production sites.

Biodiesel

Biodiesel is a vegetable oil/animal fat-based fuel that runs in current diesel engines either pure or as a blend with petrol-diesel (**Biodiesel Definition**). The most common blends are 5%, 10% and 20% biodiesel in diesel which are called B5, B10 and B20, respectively. It is formed when a triglyceride (non-polar oil) is reacted in presence of a base or acid catalyst and an alcohol. The byproduct of the reaction is glycerol. Additional byproducts are dependent on the oil source chosen for the biodiesel production. The oil sources mentioned in this report will have left over protein and some will have left over sugars that can either be sold directly or refined further. Biodiesel is formed by the transesterification of an oil with an alcohol in the presence of a base catalyst. The reaction is shown below.



Petroleum Diesel

Petroleum diesel is derived from petroleum through fractional distillation of crude oil. It has an average chemical formula of $C_{12}H_{26}$ and is composed of around 75% hydrocarbons and 25% aromatic hydrocarbons. It has high concentrations of sulfur and is less refined than gasoline.

Biodiesel vs. Petroleum Diesel

Biodiesel has several advantages over traditional petroleum diesel (commonly referred to as #2 diesel) in addition to the fact that it is an alternative source of energy. It reduces carbon dioxide emissions by 80% in B100 (pure biodiesel).

The cetane number is a factor that is used to rate the ignition efficiency of a fuel. The higher the this number is, the lower the temperature that the engine will ignite. This corresponds to a better ignition efficiency and a reduction in the “engine knock” that is associated with #2 diesel. Biodiesel has a cetane number in the range of 48-65, while #2 diesel has a cetane number between 40 and 55 (Yokayo 3). Due to this fact, biodiesel extends engine life. It has relatively no sulfur content and increases lubricity. This reduces sulfur dioxide emissions by nearly 100% and also adds to the extended engine life. Petroleum diesel has high concentrations of sulfur and lacks lubricity. The high sulfur content helps in #2 diesel’s lubricity, so lowering the sulfur content will have a negative impact on its lubricity. Petroleum diesel’s high sulfur concentrations also poisons the catalyst in the exhaust system which reduces its overall effectiveness. The flash point of biodiesel is 260ºF, while that of petroleum diesel is 125ºF. This makes it safer to store and transport biodiesel than #2 diesel (Yokayo 3). The energy efficiency ration is defined as the fuel energy divided by the total energy used in production, transportation an distribution. Biodiesel has a lower energy efficiency than #2 diesel; however, for B100 the efficiency is only 9% less than that for regular diesel.

Biodiesel is a strong solvent that can dissolve certain rubbers. Blends of biodiesel with diesel in excess of 20% biodiesel will have an effect on engines that were built preceding 1972 (Yokayo 2). Biodiesel can dissolve the hosing in those engines. For all engines that have been previously run on #2 diesel, biodiesel's solvent ability will dissolve carbon build-up and residue in the engine. This is a positive effect because it cleans the engine and helps it to run smoother. However, it is also a negative effect because the gunk and residue that is loosened can clog fuel filters and injectors (Yokayo 3). In order to prevent this scenario, a gradual switch from petrol diesel to biodiesel is recommended. A minor disadvantage of biodiesel to #2 diesel is an increase in NO_X emissions. For B100, the increase is around 10% (Source). Some modifications will have to be made in order to optimize engines running on B100. However, the advantages of biodiesel vs. petroleum diesel outweigh the drawbacks.

Oil Sources

There are several easily produced oil sources that can be used for biodiesel production. These sources include agricultural crops such as soybean, sunflower, rapeseed, canola, and corn, waste frying oils from restaurants and certain types of algae. Waste frying oils are free and in some cases restaurants will pay to have someone haul their waste oil away. While waste oil has been proven to be a profitable source due to this fact, it is not an abundant source. The limited supply of frying oils cannot support the large scale production of biodiesel. Currently there are about 60 Biodiesel plants in the U.S. in operation. Most of those use soybean as their source of oil. There is currently an incentive program in which the Comodity Credit Corp (CCC) gives 40% of purchasing costs of vegetation that is soley produced for biodiesel production facilities to help dampen the major costs of an oil source (Economics of Biodiesel). Even with these applied incentives 24% of the soybean plants are still not profitable. The land conversion of the agricultural crops is excessive and will limit the amount of production based on the limited land availability. Table XXX below compares the required amounts of land for three different crop oil sources: canola, soybean and algae for three different biodiesel blends, B20 (20% biodiesel), B10 and B5. (Widescale Biodiesel Production from Algae)

Table 1: Oil Sources

Soybean	Blend	(% Biodiesel)		B20	B10	B5
	Barrels Req.	(bbl / year)		48,829,000	24,414,500	12,207,250
	Required land	Low	(mi^2)	7,630	3,815	1,907
		High	(mi^2)	9,537	4,768	2,384
	Oil production	(gal / acre)		40 - 50		
	Crop planted	(mi^2)		625		
Canola	Blend	(% Biodiesel)		B20	B10	B5
	Barrels Req.	(bbl / year)		48,829,000	24,414,500	12,207,250
	Required land	Low	(mi^2)	2,631	1,315	658
		High	(mi^2)	3,468	1,734	867
	Oil production	(gal / acre)		110 - 145		
	Crop planted	(mi^2)		?		
Algae	Blend	(% Biodiesel)		B20	B10	B5
	Barrels Req.	(bbl / year)		48,829,000	24,414,500	12,207,250
	Required land	Low	(mi^2)	19	10	5
		High	(mi^2)	76	38	19
	Oil production	(gal / acre)		5,000 - 20,000		
	Availible	(mi^2)		-		

As shown above, algae consumes significantly less land than either canola or soybean. As the desired production capacity increases, the land requirement for algae is much more feasible. With higher land consumption to plant the necessary amount of soybean or canola, there will be much more diesel consumed to grow and to transport the plants to harvesting. This will counter some of the positive effects that biodiesel has on the environment relative to #2 diesel.

Advantages of Algae

Algae require less land than traditional crops due to their higher oil yields per acre of biomass produced. To compare, traditional crops yield around 50 to 150 gallons of biodiesel per acre per year, while algae can yield 5,000 to 20,000 gallons per acre per year (**Widescale Biodiesel Production from Algae**). Furthermore, algae use carbon dioxide as a carbon source during photosynthesis. When coupled with the fact that micro algae are nitrogen fixating organisms, algae can be seen as an important means for reducing emissions. Thus, if grown near power plants, algae would be capable of reducing CO_2 and NOx emissions before the biodiesel's reduction of these emissions is considered. Algae is also not currently being grown for any other purpose, so it will not impact other markets, particularly those relating to food sources. With such high lipid yields, production cost should theoretically be lowered and this will increase the probability of making biodiesel production profitable.

Problem Statement

Large scale algae production for the purposes of a biodiesel feedstock is a relatively new technology. In order to make biodiesel from algae, the algae first have to be grown, harvested, and oil extracted. Then, the oils have to be transesterified and the various byproducts refined. Byproducts including those from the transesterification: glycerol and those from the algae: protein and sugars. Several of these processes are costly and the algae production will need to be optimized and modified for the specific location. Finally, the algal species that dominates in normal conditions will be the species chosen for genetic modification in order to maximize lipid production. A previously researched species will be chosen for a starting point and experimentation and modification will have to ensue post production.

Overall Process Design

Different procedures are explored in the biodiesel production process. These procedures include the growth of large amounts of algae, a water recycling system to maintain salinity and water levels, pond design, algae harvesting, oil extraction, transesterification of oil to biodiesel, and byproduct reformation. The role each procedure has on the overall process is shown below in Figure 1.

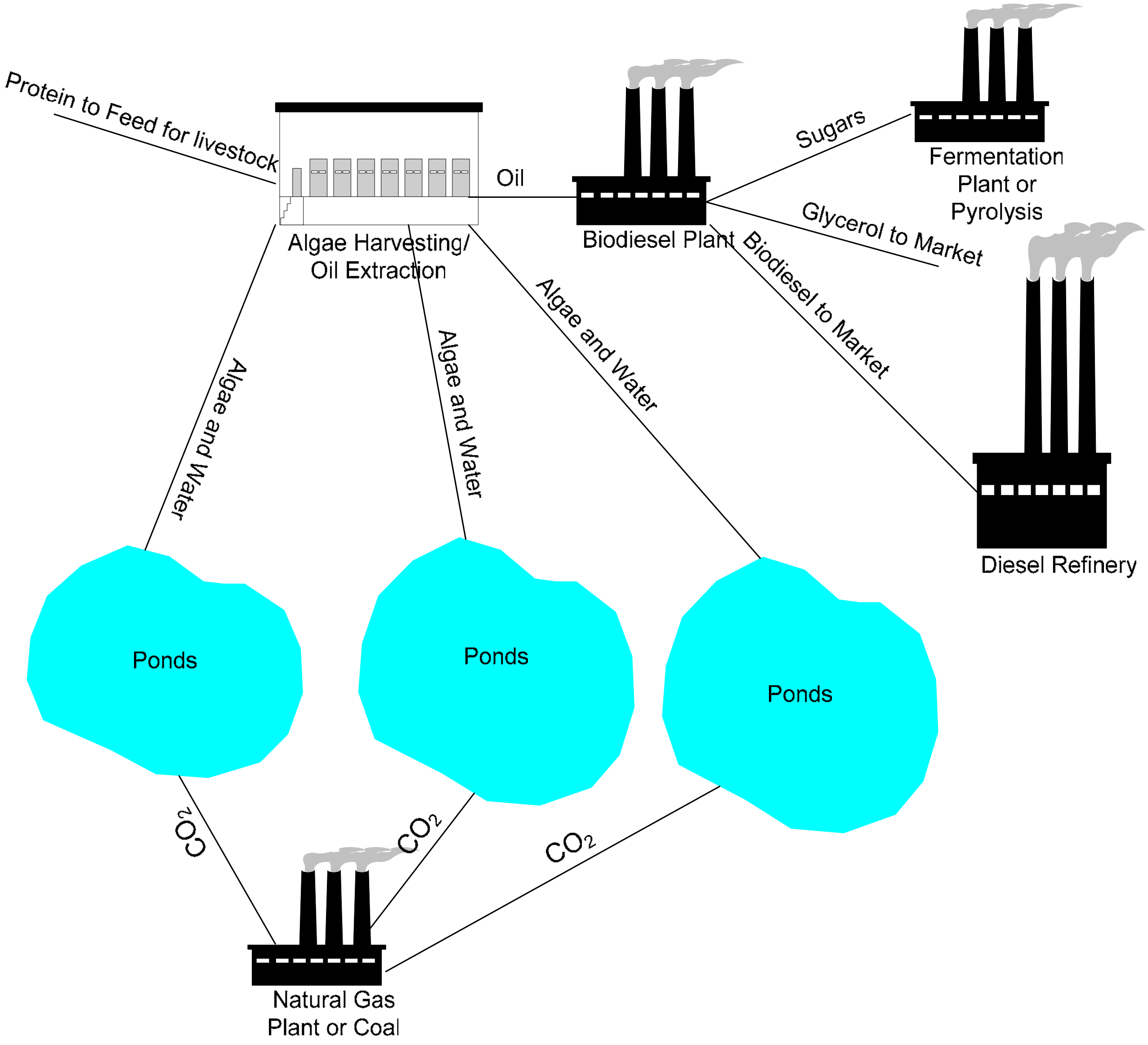


Figure 1: Biodiesel Production from Algae Process Flow Diagram.

As it can be seen above, carbon dioxide from a natural gas or coal burning plant is utilized by bubbling flue gas from plant into the bottom of the ponds. A food source is added to the water with a small amount of the chosen algae. At the seventh day the algae and water in 1/7 of the ponds is drained and sent to harvesting, where the algae is dewatered. The oil is then extracted from the algae and sent to the biodiesel plant where transesterification takes place with methanol and the base catalyst, potassium hydroxide. The remaining protein in the algae is sold as a feed to livestock and the remaining sugars are fermented to xanthan gum. Several other fermentations and the refining process of pyrolysis was explored as well. This can be found in the Byproducts section explained in further detail. The produced biodiesel is sent to a refinery where they can make a 20% blend of biodiesel in petroleum diesel, B20. The glycerol byproduct of transeseterification can be sold to the cosmetic industry at approximate 80% purity that is attained through separation processes. The entire process has been separated into sections accordingly and each section explores the different steps in greater detail.

Plant Location

Based on the entire process described above and the needs of algae, a location was chosen for the biodiesel facility. First, the solar radiation was considered so that the algae could have the maximum amount of sunlight available for photosynthesis. The Southwestern United States has the maximum amount of solar radiation in the U.S. with energy rates of 5-6 $kWh/m^2/day$. In this region, Texas was chosen because it has 24 of the diesel refineries in the U.S. which would provide easy access to sell the biodiesel for blending purposes and 8% of the U.S. diesel used on highways and roads which will give the facility a greater potential of making a national impact on the diesel demand. Coal burning and natural gas plants were located in western Texas as possible CO2 sources. Three different plants, one coal burning and two natural gas, were evaluated based on the flue gas composition. Tolk Station is the coal burning plant and its flue gas concentration of sulfur dioxide is much higher than that of the natural gas plants, so it was eliminated as a possible source. Such large amounts of sulfur dioxide would effect the acidity and thus overall pH of the water in the ponds. Increasing the pH significantly would pose a threat to algae survival. Between the natural gas burning plants, there was information available in the literature about algae in the region of the Newman Plant than in the Jones Plant. Choice of algae is one of the key factors to the success of production and profitability of the biodiesel facility. The National Renewable Energy Laboratory (NREL) did a study on biodiesel from algae in 1984-1987 and the literature available on the different algal strands that they investigated was the main source used in determining a starting algal species for this biodiesel facility.

Algae Production Specifications

Algae need sunlight, carbon, nutrients(nitrogen or silicon), water, and optional circulation or aeration for survival and growth. The CO_2 in the flue gas is the carbon source, sunlight is readily available in the chosen location, urea is added as a nitrogen source, and the circulation is achieved by the use of paddle wheels in raceway ponds which will be discussed further later in this report. Algae is harvested from each pond every seven days, at which the food source has depleted and the algae will have increased lipid contents due to this starvation.

This is for the purpose that 7 days completes the cycle, so to keep the entire process as continuous as possible, harvesting will occur everyday with 1/7 of the total ponds being harvested per day. The algae is pumped out of the ponds with multiple pumps over night and sent to the harvesting plant where dewatering occurs before the oil can be extracted. As the algae is dewatered the water is pumped back to the ponds and an approximate percentage of this water is removed and replace to balance the salinity to keep a consistent environment for the algae. A great amount of evaporation occurs in the Newman Plant area. This causes an additional increase in the salinity of ponds. The large amount of water that is available for the ponds is from an aquifer that is a great source of brackish (saline) water. The very saline water is disposed of and replaced with reclaimed water. The following sections will go into the basic steps in the pond maintenance and algae growing process.

Water Source

The primary source for our water is the Hueco Bolson Aquifer which supplies water to the El Paso region. The Aquatic Species Program from the NREL Study focused on the production of biodiesel from algae. Arizona State University collected the strains of algae in the Newman Plant and Western Texas region. These strains were tested in different media. The main difference in media that was focused on was salt concentration. However, algae choice will be discussed in a later section. The aquifer is used as a fresh water source for the city of El Paso. It contains 600 times the amount of brackish water as fresh water (**Brackish Water**). The brackish water is located below the fresh water. The brackish water in the aquifer at the Newman plant location has a salt concentration range of 1000-3000 mg/l. This water will be used to fill the ponds initially which requires 1 billion gallons of water for 7 square miles. Reclaimed water will be used to maintain a balance of salinity by replacing portions of the high saline content water in the ponds after consistent operation. It is purchased at \$0.94/1000 kg from the city of El Paso (**Reclaimed Water Rates**). With low concentrations of nitrates and phosphates, 1.81 mg/L and <0.01 mg/L, the water needs supplementary nutrients (Source if found otherwise take out). The main nutrient that will be needed for our algae is nitrogen. The specific algae that was chosen is a blue-green algae that requires nitrogen for a feed. We will use urea as our nitrogen source. It will cost approximately \$130/ton and based on a max desired concentration of 144mg/l for 1 square mile of ponds will cost about \$500,000 per year (**UREA COST**). The urea will be replaced with each 7 day cycle and for 1 square mile 75 tons of urea per cycle is required. Urea is the main source that NREL used in testing the different algal strains. The 144mg/l came from the high end of the range that was used in the media in the literature (**NREL**).

Pond Design

The ponds are oblong in shape and were based off of the NREL design of raceway ponds. Raceway ponds are oblong in shape and have a divider down the center of the them with a paddle wheel positioned on each side of this divider. The paddle wheels are to aerate the algae and to evenly distribute sunlight to the algae. The arrangement of 8 ponds in 1 square mile is shown in Figure 2 below.

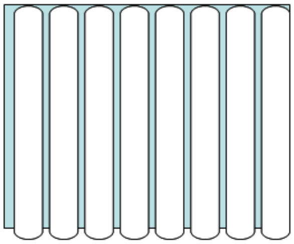


Figure 1: Pond Design

The dimensions above, 1835 m in length and 179 meters in width.

Growing Cycle

In the interest of maintaining a semi-continuous process, a seven day growing cycle for 8 ponds/square mile was chosen. Eight ponds was chosen because it is a cyclic process in which one pond per square mile is drained and sent to harvesting. As the water is being removed from the algae it is returned to the 8th empty pond so that there will not be disruption in the other ponds still growing algae. Considering the algae starvation process, it would be best if there are at least 7 ponds or multiples of 7 due to the 7 day growing cycle. If there are fewer than 7 ponds, then a portion of a pond will have to be drained each day and this would disrupt the best conditions for the algae growth and lipid production. During production, the ponds are drained at night, filtered, and the water is returned to the ponds by daylight, therefore maximizing utilization of sunlight. Total time to drain the ponds and return it will take 12 hours. All nutrients and water is added at the start of the cycle, while being fed carbon dioxide continuously. Towards the end of the growing cycle the algae consumes most of the nitrates, then starts to starve, and thus increase the percent of lipids.

Salinity

Maintaining the salinity of the system is challenging with many changing conditions. With the amount of rainfall over the course of the seven day growing cycle, the salinity of water from the aquifer, and the amount of evaporation, it is possible to use a simple mass balance to calculate the amount water that must be replaced with well water to maintain the uniform salinity in the pond water. During the summer months, in the desert of El Paso, in a cycle of seven days, the amount of water in the ponds will decrease by a varying, but determinable percentage. After filtering the water from one pond to extract the algae, this percent of the total water is dumped into an evaporation pond. Since the evaporation ponds are have to evaporate fifteen percent of the total volume of one pond in one day, they have to be extremely shallow. The evaporation rate from the evaporation pond is much higher, due to solar radiation penetrating much further and heating the ground below. Therefore, the size of evaporation pond required is thirty-two percent of the total land requirements. After the water has evaporated, a mixture of different salts is left which must be physically removed and disposed of.

Alternatively, the excess brine water can be reinjected below the five hundred foot well to 1,500 feet. Although this option is probably more economical, it would require our facility to remove any excess nutrients added to the ponds, and obtain a permit from the EPA. A proposed desalination facility located only few miles from the proposed site has been in legal litigations since at least 1997 to obtain the rights to reinject three million gallons per day brine waste. El Paso Water Utilities (EPWU) was forced to commission a US Geological Survey to determine the type of rock that they would be pumping into and its effect on the aquifer. Therefore, in the interest of time to construct the algae farm, reinjection was not investigated further.

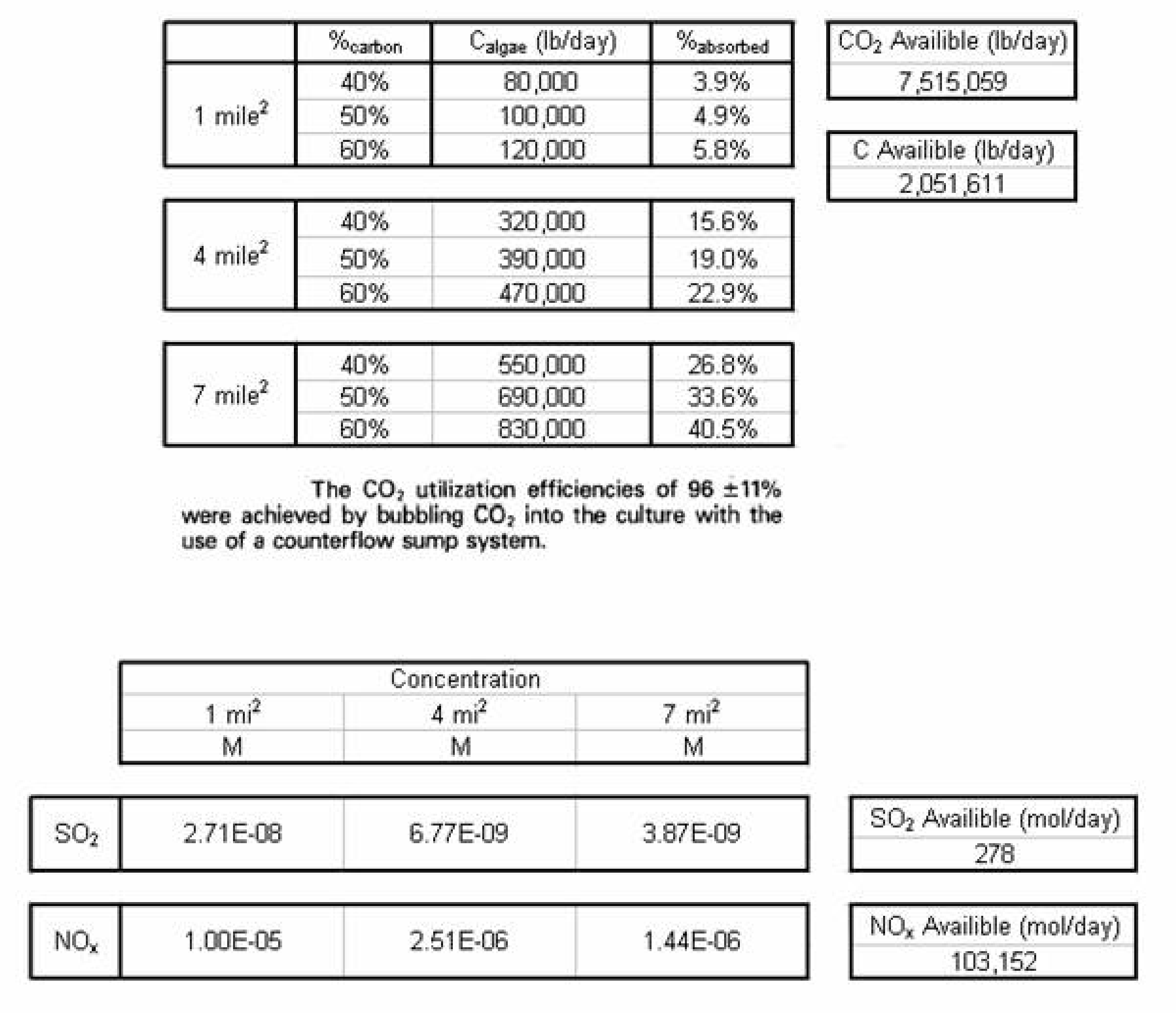
Brine Waste Management

The amount of solid brine waste produced per year was calculated by multiplying the total amount of dissolved solids present in the replacement water, 330mg/L, by the volume of replacement water. This is based on the assumption that the system is at steady state. Since the one square mile basis produces four tons of solid waste per year and the seven square mile produces twenty-six tons per day, neither was considered small enough to be moved by an operator. Therefore, a tractor was determined necessary at any flow rate. The smallest tractor that would be considered dependable was a few year old Bobcat manufactured by Caterpillar, for thirty-two thousand dollars. [5] The tractor is capable of lifting 1950lbs at a time, so it is more than able to move four tons or twenty-six tons. The cost estimated to dispose of this solid waste was estimated at thirty-eight dollars a ton.

SO2 and NO$_X$

When deciding a plant location, SO_2 and NO_X in the flue gas were considered to have possible negative effects on the acidity of the pond water and overall pH. It was assumed that, all the sulfur dioxide under the presence of the catalytic nitrous oxides would convert to sulfur trioxide, which in turn would react totally with the water, effectively assuming one hundred percent yield. It was also assumed that none of the sulfuric acid would be evaporated, the water was totally unbuffered, and even the small ponds would have all the flue gas bubbled through. Therefore, daily 7.61 kg of sulfuric acid react with a minimum of 12.4 million kg of water at a pH of 8.02. [3] [4] Hence, there is no change in pH that will affect the algae. NO_X on the other hand tends to benefit the algae. Depending on the amount of UV exposed to the nitrous oxide, NO_X will form a combination of nitrates and nitrites. Nitrites are consumed by bacteria that live naturally in the water to produce nitrates. As for the nitrates, most aquarium owners know excess nitrates cause algae blooms.

Table 2: Flue Gas Components



	$\%_{carbon}$	C_{algae} (lb/day)	$\%_{absorbed}$
1 mile^2	40%	80,000	3.9%
	50%	100,000	4.9%
	60%	120,000	5.8%
4 mile^2	40%	320,000	15.6%
	50%	390,000	19.0%
	60%	470,000	22.9%
7 mile^2	40%	550,000	26.8%
	50%	690,000	33.6%
	60%	830,000	40.5%

CO_2 Availible (lb/day)
7,515,059

C Availible (lb/day)
2,051,611

The CO_2 utilization efficiencies of 96 ±11% were achieved by bubbling CO_2 into the culture with the use of a counterflow sump system.

	Concentration		
	1 mi^2	4 mi^2	7 mi^2
	M	M	M
SO_2	2.71E-08	6.77E-09	3.87E-09
NO_x	1.00E-05	2.51E-06	1.44E-06

SO_2 Availible (mol/day)
278

NO_x Availible (mol/day)
103,152

Piping

The size of pipe used to transport the CO_2 to the ponds and to drain the ponds in the given ten hours was chosen based on the maximum velocity. The optimal velocity for water in a pipe is usually around three meters per second, and with the extremely large volume of the ponds, the resulting pipe diameter is large. In the case of a one square mile basis, the required pipe diameter is approximately four feet. Since there is no need to pump carbon dioxide to a pond while it is being drained, the pipes will carry carbon dioxide to the pond as well as moving the algae water to the plant. In order to prevent back flow laying the pipes above the high of the ponds is necessary. Therefore, in order to keep down the costs and to ease installation, high density polyethylene (HDPE) was chosen for the pipe material. The benefit for using HDPE extends beyond costs. HDPE piping is resistant to corrosion caused by fluctuations in pH, salinity and temperature making it a perfect candidate.

Once again in order to maximize the use of the equipment the pumps were used for two purposes. In their first roll, the pumps have to move water from five hundred feet below to the respective ponds. In the second roll the pumps have to move the total volume of one pond to the plant and onto an empty pond all in ten hours. For the one square mile case, these pumps are required to move approximately 81,000 gallons per minute. In addition, since the pumps have to move salt water thick with algae and sewer waste, they have to have special design considerations. Hence a series of high volume pumps that can be run in either parallel, for the case of moving the algae, or in series, in the case of pumping water from the well were required. Type NCD-17 Goulds sewage pumps were chosen because they are available used, and have the required flow rates.

Algae Harvesting

Amphora Bacillariophyceae and *Oscillatoria Cynaophyceae* were chosen as starting cultures due to their effectiveness in research studies. *Amphora*, a diatom that exhibits a growth rate of 0.90 doublings per day, was confirmed to have the highest amounts of oil content, 551mg/l triolein (triglyceride). In addition, it exhibited a particle size of 10 X 4 microns. On the other hand, *Oscillatoria* is a blue-green algae that is much more adaptive towards different salinities regions than *Amphora*. However, *Oscillatoria* is much smaller with dimensions of 0.2 X 0.1microns. These small dimensions make it difficult and costly to harvest the algae and separate them from the pond water. Realistically, one of these algae species is chosen to begin production and experiment upon to optimize culture growth and lipid production. In the initial start-up of the facility, the algae will have to be monitored and determined to be fit for the environment. If a native algae takes over and survives much better, then this algal species will have to be simultaneously studied and experimented with in order to maximize both growth and lipid content.

There are several different options for separations, which include centrifuging, flocculation, and filtration. Flocculation requires an additive known as a flocculant to be added to the algae so that it will either rise to the surface of the water or fall to the bottom of the water in order to ease separation of the algae from the water preceding oil extraction. All of these processes are costly due to the large throughput of 90 to 630 tons of algae per day. The following table lists the range of prices for each type of separation and their respective throughput for algae(Becker 161-163).

Table 3: Compared Separation Costs per kilogram

	Cost($/Kg)
Centrifugation	1.71
Filtration (belt)	0.5
Flocculation	1.39

The algae used for production will be determined by the dominate species in the pond environment that will be created at the biodiesel facilities. This environment encompasses the salinity of the water, the different nutrients in the water, the temperature of the water and the amount of sunlight available. The dominant species can then be genetically modified to increase or maximize lipid content. The determination of the dominant species will take an initial data collection and research period in order to verify which species dominates under various conditions. Algae selection is a large risk factor in the design of the process because of the large range of possible lipid content of the algae. Therefore, calculations were carried out for algae that have theoretical lipid contents of 20, 30, and 40%. An example for clarification would be, algae species X produces 20% lipid under normal conditions, 30% lipid under starvation from nutrients(nitrogen or silicon) and can be genetically engineered to produce 40% lipid. Exploring the lipid contents will also give a better idea of the effect the lipid content has on profitability.

The basic steps chosen to harvesting the algae include filtering with a belt filter to go from 0.04% solids in water to 4% solids in water, centrifuging from 4% solids (algae) to 60% solids, and drying to 90% solids or more. A schematic is shown in Figure 3 below.

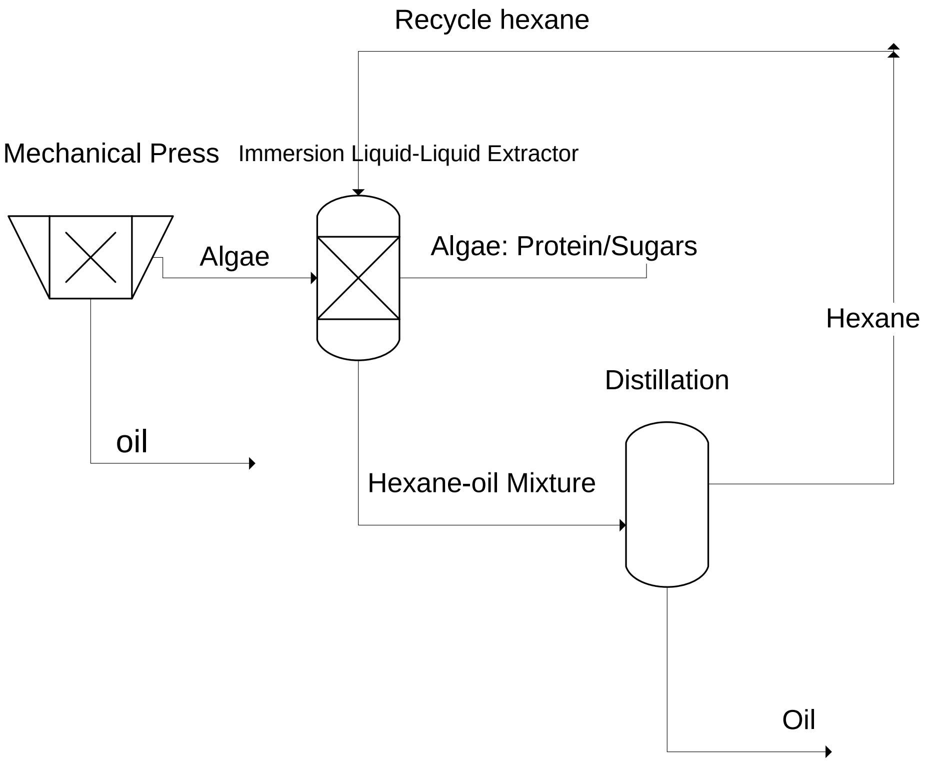


Figure 3: Algae Harvesting

The algae must have 10% or less water content so that it will not spoil or support the growth of different bacteria (Becker 165). The harvested dry algae is then sent to the oil extraction section of the plant.

A schematic of an example of belt-filtration is shown below in Figure 4.

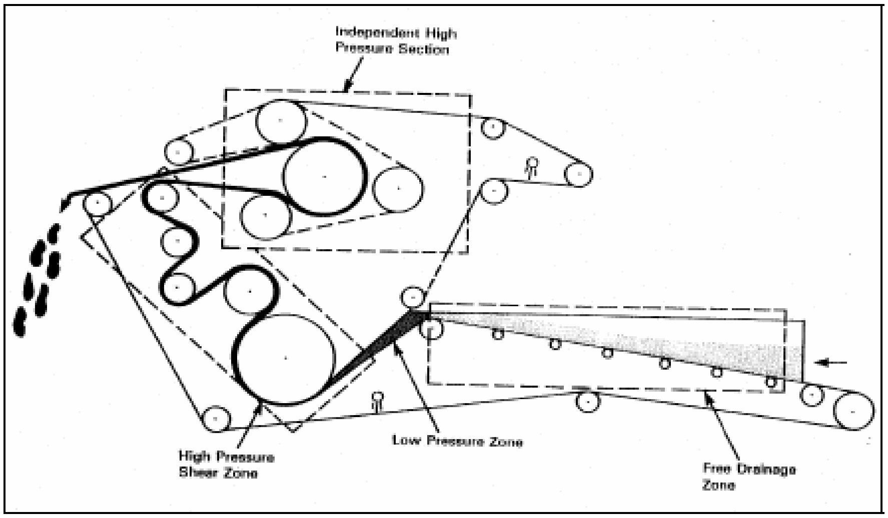


Figure 4: Belt Filtration

There is high pressure and low pressure region, however most of the filtration works on the basis of gravity and this is why belt filtration can only increase the percent solids by 100 fold.

Figure 5, below, shows the spray drying process which was chosen for drying because it is the one of the most commonly used methods to dry algae and the feed stream can be less concentrated compared to other drying processes (Becker 171).

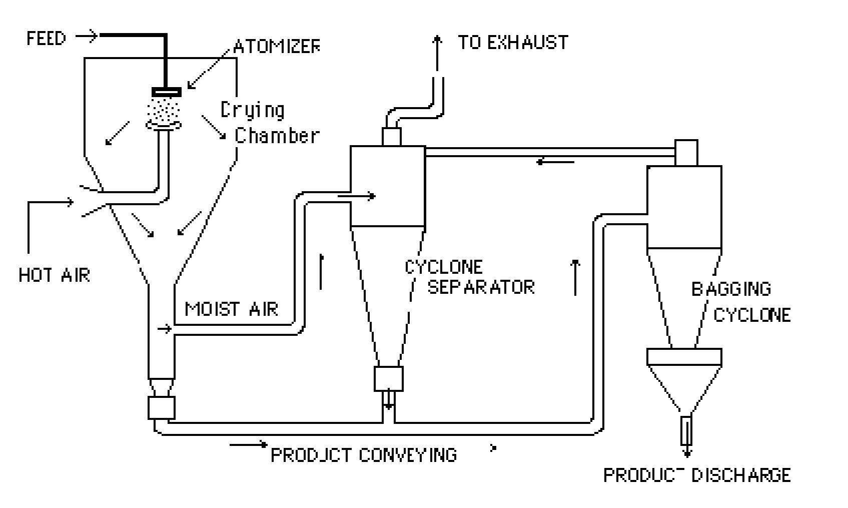


Figure 5: Spray Drying Schematic

Oil Extraction

Oil extraction has two steps: a high pressure mechanical press to obtain the first 70% of the oil and an immersion liquid-liquid extraction with hexane is used to obtain the remaining 20-30% of the oil. Using this process, a yield near 99% of oil can be extracted from the algae. A schematic of the oil extraction is shown below in Figure 6.

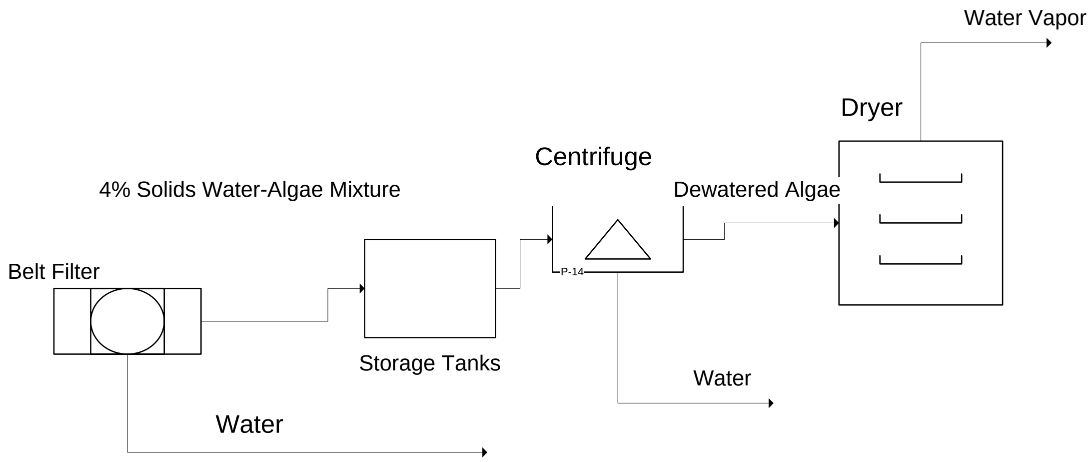


Figure 6: Oil Extraction

Hexane is used in the immersion liquid-liquid extractor (ILLE) as the solvent and the oil hexane mixture that exits the ILLE is sent to a distillation column for separation. The distilled hexane is then recycled back into the feed. As the oil is extracted, it is sent to the biodiesel plant to be converted to biodiesel via transesterification. A 60 ton-capacity wine press was used for the mechanical pressing of the algae. An example of an immersion liquid-liquid extractor is shown in Figure 7 below.

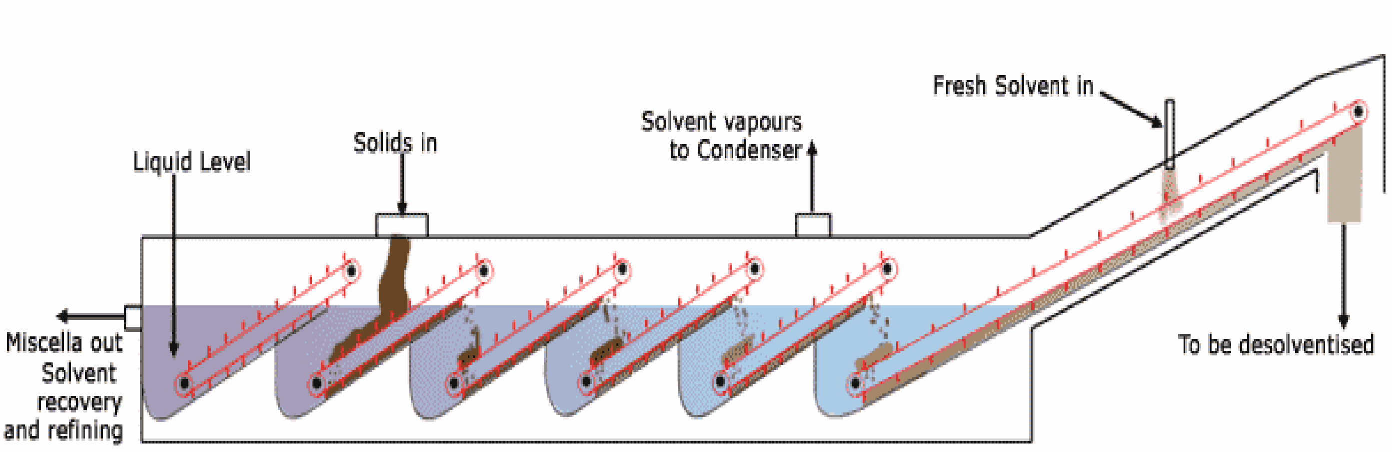


Figure 6: Immersion Liquid-Liquid Extractor

The hexane enters the extractor at the top of the extractor and travels down to the other end while contacting the algae. The algae enters the top of the extractor at the other end and travels by means of conveyors the opposite direction toward the hexane feed. The hexane-oil mixture exits the bottom end on the side from which the algae entered.

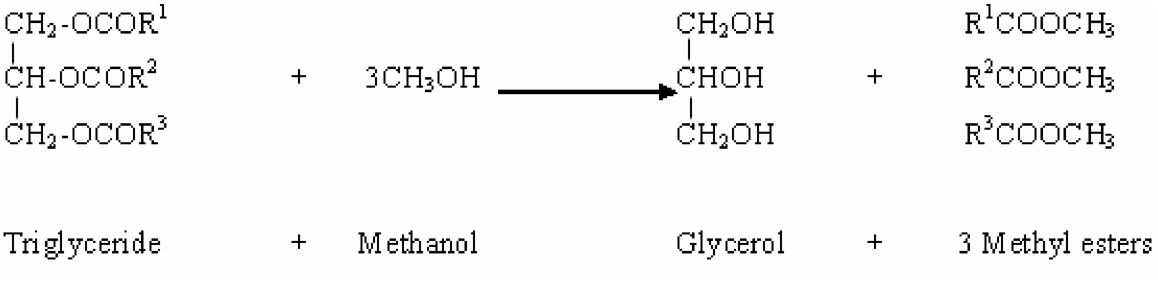
After all of the oil has been extracted, the remaining sugars in the algae are sent to the fermentation plant to produce xanthan gum and protein is sold as a feed for livestock.

Due to the algae’s small size and the large volume of water they are contained in, the algae harvesting and oil extraction processes constitute two of the most expensive factors in the production of biodiesel.

Transesterification

There are some negative side effects associated with burning raw oils in diesel engines. Burning raw oils can loosen metallic residues embedded in the engine and thus release them to into the engine. This can lead to engine knocking and eventually engine failure. It can also clog fuel filters. Therefore, the raw oils must be converted into biodiesel through a process known as transesterfication. The biodiesel produced through transesterfication may then be burned in regular diesel engines. Biodiesel burns comparable with type 2 diesel, but with 80% less CO_2 and virtually no SO_2 emissions.

Transesterification involves the reaction of a triglyceride (oil) with an alcohol in the presence of a catalyst. The reaction (**Renewable and Sustainable Energy Reviews**) is as follows:



$$\begin{array}{l}CH_2\text{-}OCOR^1\\ |\\ CH\text{-}OCOR^2\\ |\\ CH_2\text{-}OCOR^3\end{array} + 3CH_3OH \longrightarrow \begin{array}{l}CH_2OH\\ |\\ CHOH\\ |\\ CH_2OH\end{array} + \begin{array}{l}R^1COOCH_3\\ R^2COOCH_3\\ R^3COOCH_3\end{array}$$

Methanol was chosen as the alcohol to be used in the process because it is less expensive than ethanol and it makes the downstream separations simpler. If ethanol were used, downstream separations would be more difficult since ethanol tends to form stable emulsions because of its larger non-polar group. The more stable the emulsion, the less likely the esters and glycerol are to separate.(**Renewable and Sustainable Energy Reviews**) As shown in the reaction above, the required molar ratio of methanol to triglycerides is 3:1; however, a 6:1 molar ratio is used in the reactor in order to maximize the reaction yield by pushing the reaction farther to the right with an excess of methanol. Research has shown that this particular ratio is the best to reach the highest yield in transesterification. If the molar ratio is increased or decreased from 6:1 then the yield will decrease. (Biodiesel from Frying Oil)

The oil that is obtained from the algae is assumed to be virgin oil without any contaminants. Contaminants are a problem with frying oil and would require the oil to be degummed before transesterification. If the oil contains greater than 3% of free fatty acids (FFAs), then an acid catalyzed reaction will have to be used in the biodiesel production (**Renewable and Sustainable Energy Reviews**). FFAs decrease the yield of biodiesel during transesterification.

Sodium hydroxide was chosen as the base catalyst, since is relatively abundant, inexpensive, and can be interchanged with potassium hydroxide. This is convenient especially in the event that one of these bases becomes much more inexpensive than the other. In order to maximize conversion, 1% catalyst was used, however, if too much catalyst is added it can contribute to the formations of emulsions, making separations more difficult and reducing the reaction yield(Biodiesel from Frying Oil).

The process for the Biojewicz Biodiesel Production Facility was modeled after a base catalyzed reaction from the BioResource Technology Journal (**A process model to estimate biodiesel production costs**). Figure 7, below shows the flow diagram for the process.

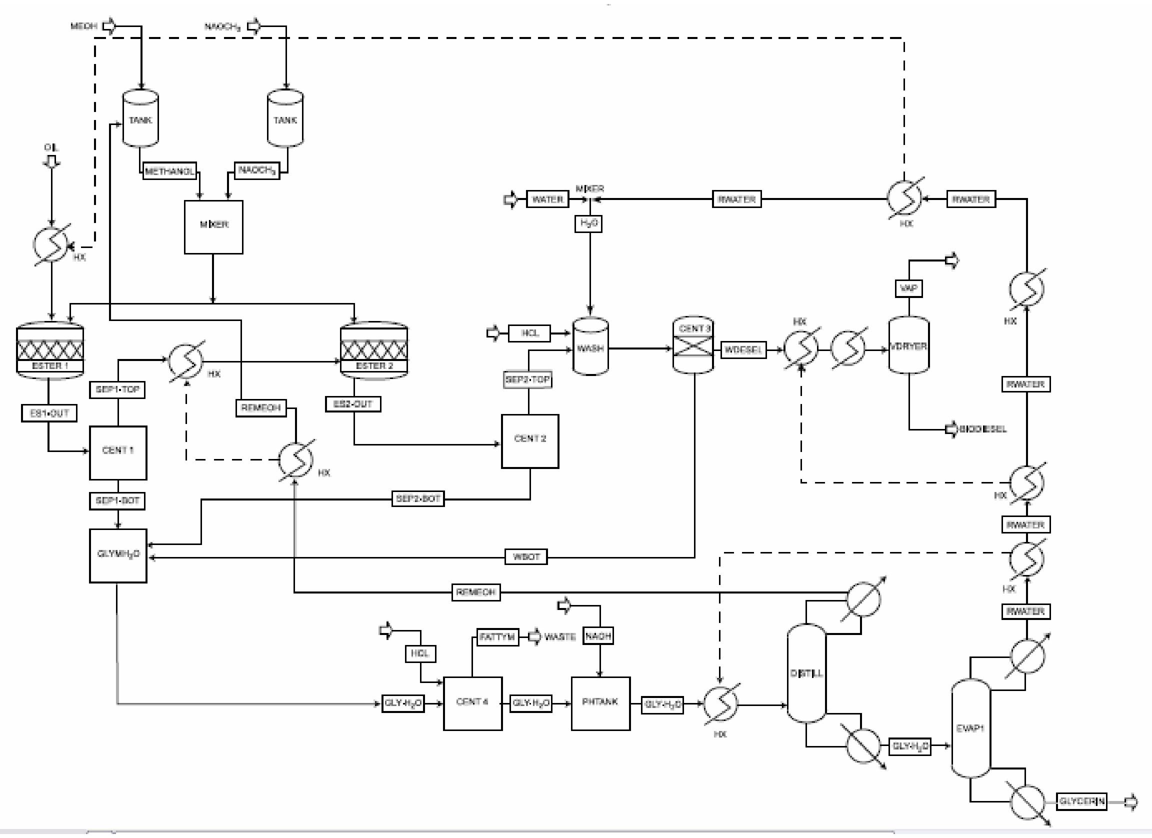


Figure 7: Base Catalyzed Transesterification (A process model to estimate, etc)

The process consists of two CSTRs in series, with each assumed to operate at 90% efficiency, thus resulting in an overall efficiency of 99%. The product stream exiting each reactor is then centrifuged to separate the glycerol from the methyl esters (biodiesel). The glycerol and a portion of the methanol exit the bottom of the centrifuges and are combined prior to entering a third centrifuge, where hydrochloric acid is added to convert soaps to fatty acids and the fatty acids are removed.

The process stream exiting the reactor contains water, methanol and glycerol. After being run through a pH adjustment tank, the methanol is removed and recycled via distillation, while the water is recycled with an evaporator. The glycerol product stream, assumed to be 80% with the separations in the literature process model, can then be sold as a raw product. The biodiesel (methyl esters) and the remaining un-reacted triglycerides and methanol exit through the top of the centrifuge following the second reactor. This process stream is then washed with water and HCl to remove any fatty acids. Afterwards, the water content in the biodiesel is reduced with an evaporator.

Many different factors affect the reaction explained above, for example, the kinetics of the overall reaction includes three reversible competing reactions. Therefore, the flow rates of the process streams were approximated using the 90% conversion basis discussed above and a mole balance was generated to determine the amount of product, un-reacted material, and excess methanol in the product streams from each reactor.

For example, the first reactor contains un-reacted methanol, triglyceride, biodiesel, and glycerol. Using the balance based on conversion, the exiting stream contains the following:

$$\begin{array}{ccccccc} (TRI)*X & + & (TRI)*(1-X) & + & 3*(TRI)*X & + & (6*(MeOH)-(TRI)*X*3) \\ \Downarrow & & \Downarrow & & \Downarrow & & \Downarrow \\ \text{Glycerol} & & \text{Triglyceride} & & \text{Methyl Ester (Biodiesel)} & & \text{Methanol} \end{array} \qquad (1)$$

For each separation in the centrifuges, distillation column, and evaporators a factor of efficiency was applied to the top and bottom exiting streams. For the centrifuges, certain products were assumed to exit entirely though the top (methyl esters) or bottom (glycerol). This assumption is reasonable because of the large density difference between glycerol and biodiesel, in addition to the fact that they are immiscible in each other. The flow rates were then converted to a volume and mass basis and these rates were used to size the centrifuges and reactors.

The extent of reaction kinetics were evaluated to verify this conversion. The following three reactions compose the overall transesterification reaction:

$$Tri + MeOH \Leftrightarrow Di + R_1$$

$$Di + MeOH \Leftrightarrow Mono + R_2$$

$$Mono + MeOH \Leftrightarrow Glycerol + R_3$$

Where,

Tri=triglyceride

Di=diglyceride

MeOH=methanol

Ri=methyl ester of reaction i

Gl=glycerol

The mole balances on triglyceride, diglyceride and monoglyceride were generated and then Excel solver was used to equate the sum of the equations to zero. This was done to push the reaction to equilibrium and get the maximum yield. The extents of reaction are that solver generated are shown in Table 4 below.

Table 4: Extent of Reaction

ξ1	ξ2	ξ3
0.65146357	0.69071515	0.78492311

Using these values and the mole balances, the final concentrations of each component was determined and then the conversion, X, was calculated as follows:

$X=(F_{Tri0}-F_{Tri})/F_{Tri0}$ ()

The cost evaluation of the transesterification was approximated using the cost worksheets given in the model. The *six-tenths factor rule* was used to determine a relative cost of equipment (Peters-Timmerhaus 242). It can be used below a 10-fold capacity range. The equation:

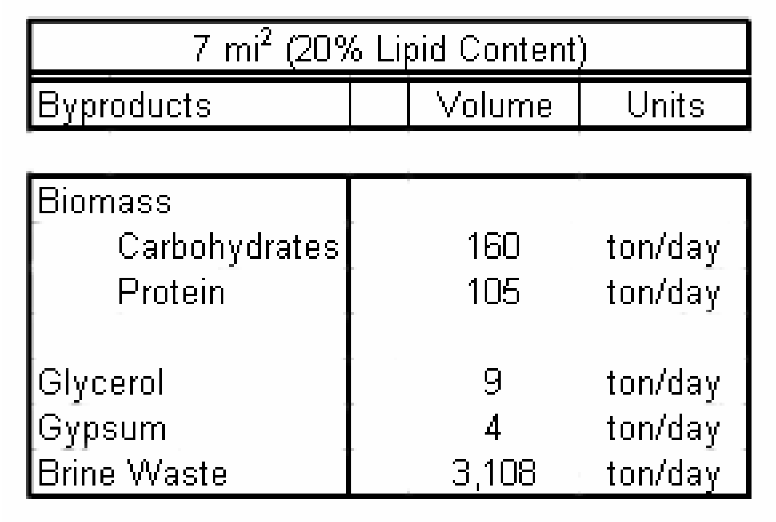
$$\text{Cost of equipment } a = (\text{cost of equipment } b)X^{0.6} \qquad ()$$

Where,
X=the ratio of the capacity of of equipment a to b

Byproducts

A large volume of waste byproduct streams are created throughout the biodiesel process, with the main byproducts being glycerol (1.8 to 3.6 tpd per mi^2 of ponds) created during the transesterfication of triglycerides and biomass (54 to 72 tpd per mi^2 of ponds) left over after triglyceride extraction from the algae. In order to maximize the overall productivity of the facility, the byproducts are going to be converted into more economic forms whenever possible.

Table 5: Byproducts generated throughout the overall biodiesel production facility.



7 mi^2 (20% Lipid Content)			
Byproducts		Volume	Units
Biomass			
Carbohydrates		160	ton/day
Protein		105	ton/day
Glycerol		9	ton/day
Gypsum		4	ton/day
Brine Waste		3,108	ton/day

There are two common methods used in order enrich biomass: pyrolysis and fermentation. Stated roughly, pyrolysis is the process used to break down organic compounds by heating in the absence or presence of oxygen, whereas fermentation is the conversion of sugars to various products through bacterial metabolic processing.

Biomass Enrichment: Pyrolysis

Typically the pyrolysis of biomass is a common method used in order to process a variety of soft and hard woods; these woods contain, to various degrees, a compound known a lignin. Lignin which “makes up about one-quarter to one-third of the dry mass of wood” (**Lignin**) fills the spaces between the various components of the cell wall and provides structure to the cell in the form of mechanical strength.

A brief study was conducted with respect to the use of pyrolysis as a means of disposing of the large amounts of biomass created during the algal production. The resulting analysis concluded that pyrolysis produces, to varying degrees based on the biomass composition, three main components: a char, flu gas, and bio-oil.

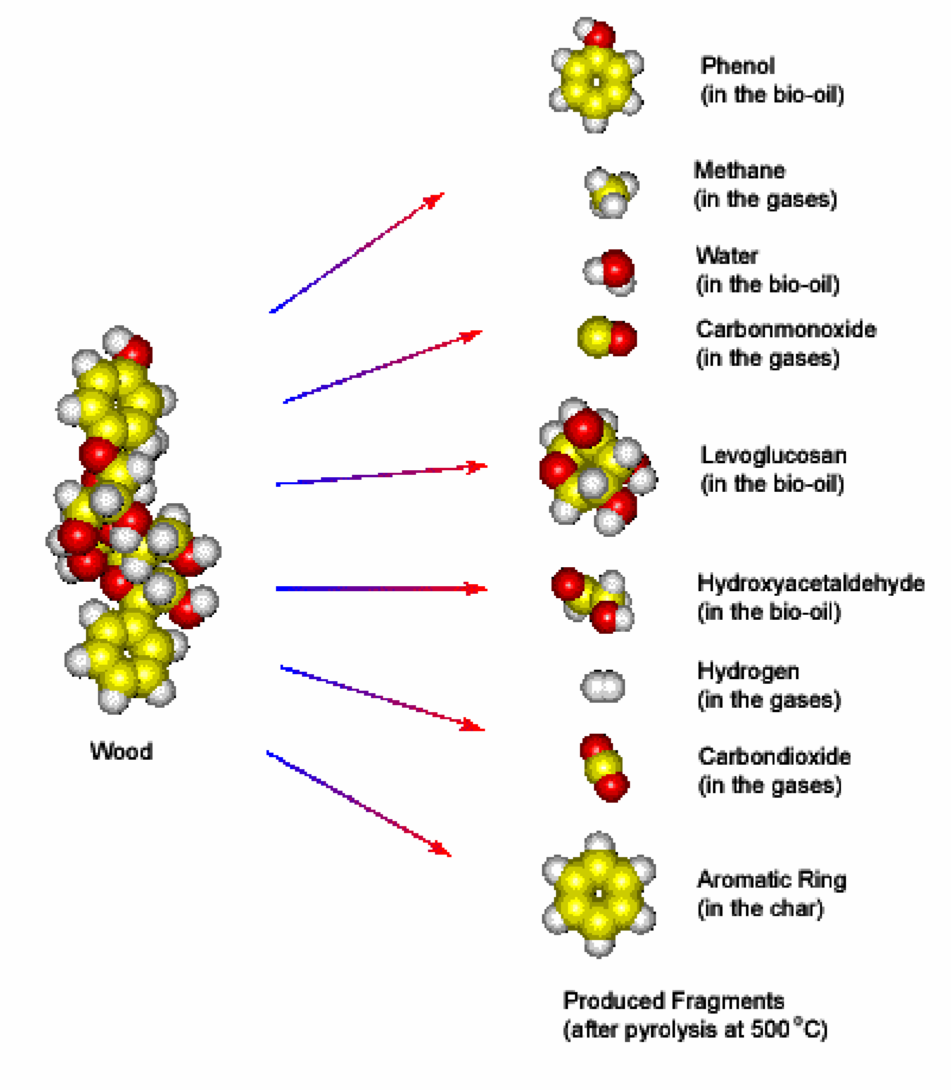
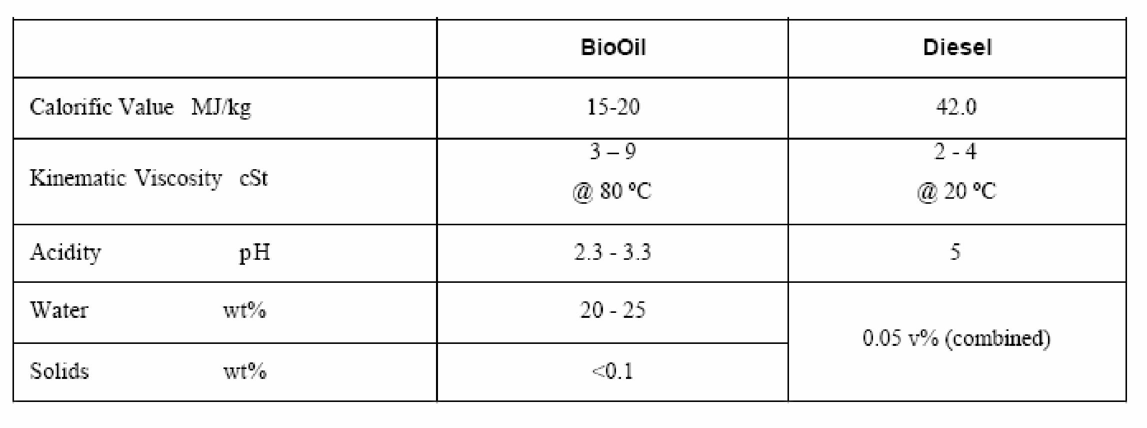


Figure 8: Typical reductions observed as wood is pyrolysed at 500 ^{o}C (Pyrolysis).

These compounds are formed as the biomass is rapidly heated to temperatures greater than 450^{o}C. The resulting pyrolysis oils, bio-oil, can be condensed and sold. Meanwhile, the char and flu gases (composed primarily of methane, carbon monoxide, hydrogen, and carbon dioxide) that are produced are generally burned as process heat, in order to reduce the heat requirement of pyrolysis process. However, the char can be developed to produce activated carbon through a process using steam or acid (**Char**). The resulting activated carbon can then be sold for use in various filtration processes.

Table 6: Property comparison between bio-oil and diesel (Pyrolysis Comparison).



	BioOil	Diesel
Calorific Value MJ/kg	15-20	42.0
Kinematic Viscosity cSt	3 – 9 @ 80 °C	2 - 4 @ 20 °C
Acidity pH	2.3 - 3.3	5
Water wt%	20 - 25	0.05 v% (combined)
Solids wt%	<0.1	

Bio-oil can be used in place of fossil fuels to generate heat and power. However, it has only been evaluated with regard to short-term applications using boilers and furnaces. Furthermore, bio-oil has not yet been applied as an automotive fuel source (**Bio-oil Applications**). Aside from its limited applicability, bio-oil has other undesirable characteristics. Bio-oil only burns at half the heating value of diesel due to its high water content. In addition, it is an acidic fuel source, so retrofitting would be required in order to avoid corrosion. However, on the plus side, bio-oil is considered a CO_2 neutral fuel source. “The carbon dioxide in these emissions is not considered to increase the amount of greenhouse gas in the atmosphere because the carbon dioxide was removed from the atmosphere by plants within the very recent past as part of the natural global carbon cycle.” (**CO2 Neutral**) It is also a viable alternative fuel source since it is naturally a liquid; therefore it can easily be transported or stored with far less risk than gaseous fuels such as hydrogen.

However, the low heating value of the resultant bio-oil in addition to its questionable market and undesirable properties…... With algae varying dramatically from what is thought of as traditional pyrolysis biomass, the resultant product concentrations of its pyrolysis may vary dramatically, resulting in a substantially different composition of bio-oil (**Pyrolytic Behavior**) than is achieved with the pyrolysis of various soft and hard woods.

Biomass Enrichment: Fermentation

The other major pathway for conversion of biomass is fermentation. In a general sense, fermentation is the conversion of sugars to various products through bacterial metabolic processes. Biomass is made up of three major components, carbohydrates, proteins, and lipids. In this case the lipids have already been removed and the carbohydrates and proteins are left behind. The carbohydrate portion (70% of lipid free biomass) of the biomass can then be isolated and fermented, whereas the protein fraction (30% of lipid free biomass) is generally sold as an animal feed source (Becker 178).

I. Two Stage Dilute Hydrolysis (subsection)

Since the leftover biomass is going to be fermented, it only makes sense to take advantage of the carbohydrate content contained in cellulose and starches that are generally un-fermentable by most bacteria. Therefore, the biomass is subjected to a dilute acid hydrolysis, a process that cleaves long chained carbohydrates into their monomer components. This process is illustrated in Figure 9.

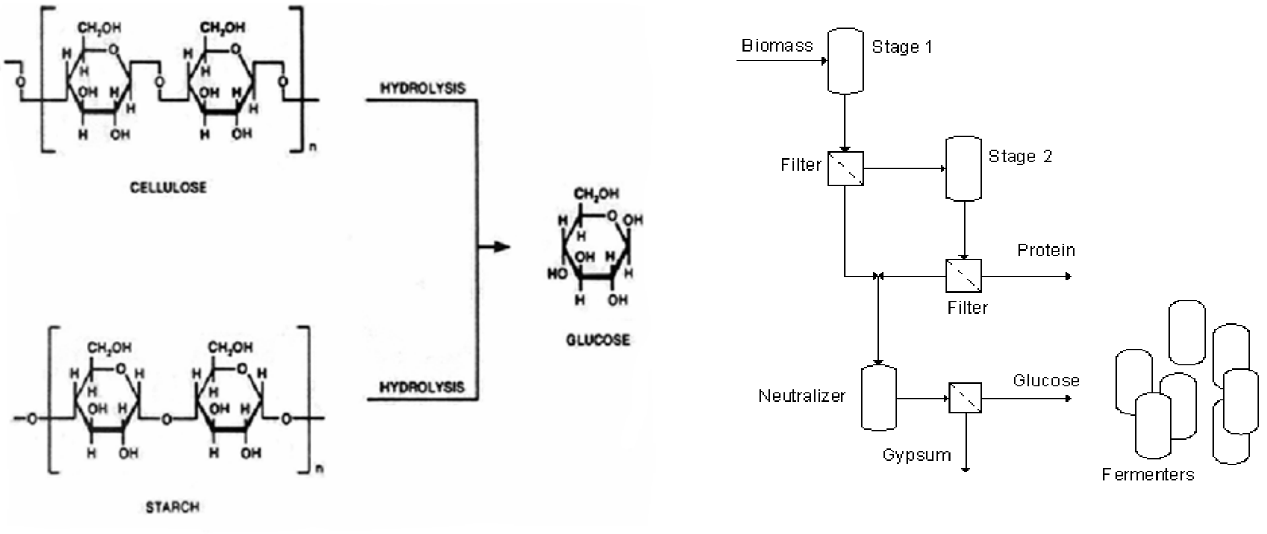


Figure 9: Hydrolysis reaction (left) 2 stage dilute acid hydrolysis process flow diagram (right)

This occurs in two stages in order to accommodate for the difference between cellulose and hemi cellulose, as can be seen in Figure 9 (right). The first stage, which specifically targets the degradation of hemi cellulose, is operated at 190°C with 0.7% sulfuric acid, while the second stage that targets cellulose and starch is conducted at an elevated temperature of 215°C with only 0.5% sulfuric acid (**Acid Hydrolysis**). These operating conditions were determined by that Department of Energy to be the optimum conditions in order to break the targeted constituent in each reactor.

As stated above, fermentation refers to the generation of various products from the conversion of sugar by bacterial metabolic processes. Fermentation products vary dramatically from organism to organism. For example, *Aerobacter aerogenes* ferments glucose to 2, 3-butanedoil, while *Streptococcus zooepidemicus* ferments glucose to hyaluronic acid and lactic acid. In addition, different bacteria require different environments in order to grow properly. Bacteria classified as aerobic require the presence of oxygen in order to carry out their fermentations, while anaerobic bacteria can ferment in the absence of air. Other factors such as salinity, pH, and media choice also play an important role in the bacteria's ability to ferment sugars.

With extremely large fermentations being carried out, it is vital to keep the system sterile. If a foreign microbe were allowed to take advantage of the sugar rich media and propagate the whole batch would be considered a failure, unless of course the alien microbe fermented the desired product to a greater extent, which is unlikely. Therefore, steam is used extensively as a sterilizing agent. All valves are under a constant steam exposure and any pipe that moves the fermentation media to or from the fermenter is filled with steam when not in use, thus keeping the environment sterile. In addition, the selection of valve type is important. A valve is sought that has little risk of harboring un-sterilized media. Therefore, valves that expose a hidden side that is generally unexposed aside from when restricting flow are avoided due to the difficulty associated with sterilizing the hidden side. Aside from the valves, it is important to keep air lines sterile, even in the event of compressor failure, so that only the desired fermentation is taking place. Therefore, as a preventative measure the sterile air feed is pumped from the ground, all the way to the top of the fermenter, and then back down and into the system.

Thus, if the compressor were to fail the media wouldn't escape into the air feed and contaminate the sterilization system.

Mixing plays an important role within the fermentation process; it facilitates both heat transfer and aeration. Even though the fermenter is jacked with either cooling or heating fluid, depending on the bacteria's preference, it is important that the temperature gradient within the fermenter is minimized. Mixing also ensures that nutrients are properly suspended within the fluid, available in concentrations desired by the bacteria for optimal sugar conversion.

Aside from the fermenter design, it should be noted, that while looking for the most economic way of enriching the biomass's value, many types of fermentations were considered. Fermentations by both aerobic and anaerobic bacteria were evaluated, with back of the envelope calculations narrowing down the wide array of possible fermentations, those fermentations that developed commodities of little value or that had limited demand were rejected, while the remaining fermentations were subjected to further analysis. General information of some of the fermentations explored can be found on Figure 10.

Succinic acid
59 hr fermentation
0.105 kg glucose/L
54 fermenters at 7 mi^2

2,3-Butanediol
0.195 kg glucose/L
35 fermenters at 7 mi^2
390 tons at $1.98/kg

Propionic acid
148 hr fermentation
0.02 kg glucose/L
115 fermenters at 7 mi^2

Butyric acid
48 hr fermentation
0.016 kg glucose/L
145 fermenters at 7 mi^2

Figure 10: Fermentations that were looked into but not pursued.

$$\frac{(kg\_glucose/day)(\frac{hrs\_fermented}{24hrs})}{1000(kg\_glucose/L)(m^{3}/fermenter)} = \#\_fermenters$$

Figure 11: Equation used to calculate the number of fermenters required.

Since many of the fermentations are longer than a day there would be a build of multiple days' sugars as the fermentations are completing. In order to ferment the amount of sugars that would build up over this period a very large number of fermenters would be required. Therefore, most of the above fermentations were rejected due to the incredibly high amount of fermenters required to use all of the glucose that is being produced. They were rejected since the large number of fermenters results in high capital cost in addition to having to find a facility large enough the numerous fermenters.

Xanthan Gum

Xanthan gum was selected as the desired fermentation product due to its elevated selling price, $11.00/kg, and since it is a very versatile product with a large market demand. The xanthan gum market is on the scale of 40 to 50 million tons per year (**Xanthan Gum Market**), with markets in many industrial applications. Thus, there is stability in the production of xanthan gum. For example, if a new technological advancement is made in the cosmetics industry and xanthan gum is no longer used as prevalently, there is still a market in many other industrial applications. The xanthan gum market breakdown more specifically is as follows:

The food industry uses xanthan gum for both its thickening properties and its ability to suspend compounds in solution. Xanthan is commonly used in sports drinks as a means of developing texture or "mouth feel" within the product; this takes advantage of the thickening properties of the gum which effectively increase the viscosity of the solution, thus giving it more of a texture. In addition, xanthan is added to frozen foods as a preventative action against freezer burn. Frozen foods contain water; however, the inside of a freezer is a very dry environment. Thus, the water attempts to diffuse from the areas of high concentration to areas of lower concentrations. Adding xanthan to acts as an emulsifier and therefore limits waters ability to diffuse through the product.

The consumer / industrial sector uses xanthan gum in a multitude of applications. It is used in cleaners, oral care products, paints, cosmetics, pharmaceuticals, and in printing. Its thickening properties minimize bleeding of paints and color additives, thus toothpaste can contain swirls. It is also used in the pharmaceutical industry for its role in time release of drugs and as an anti-abuse agent.

The oil industry uses xanthan gum in its mud's in order to take advantage of its pseudo-plastic properties. As the mud is pumped down hole the shear rate increases. This decreases the viscosity of the fluid, making it easier to pump, however, when it reaches the bottom of the well and turns 180^o to be pumped to the surface, the shear rate drops and the solution thickens. In the process all the debris generated during drilling is suspended in solution and easily removed from the hole.

Fermentation of xanthan gum is carried out by a Gram-negative bacterium known as *Xanthomonas campestris*. For optimal yields, *Xanthomonas campestris* prefers temperatures around 35^oC, with an aeration rate of 1 vvm, and higher mixing speeds around 600 rpm. In addition, the fermentation media is initially set to a pH of 7, but allowed to vary uncontrolled during fermentation during which time it rises to a pH of 9. The graphs in Figure 5 below represent the production and consumption of products and reactants over the course of the 72 hour traditional fermentation with an initial glucose concentration of 2 g/L; however, the glucose concentration was increased to 50 g/L in the industrial scale up so that the reaction would not be limited by the amount of glucose substrate.

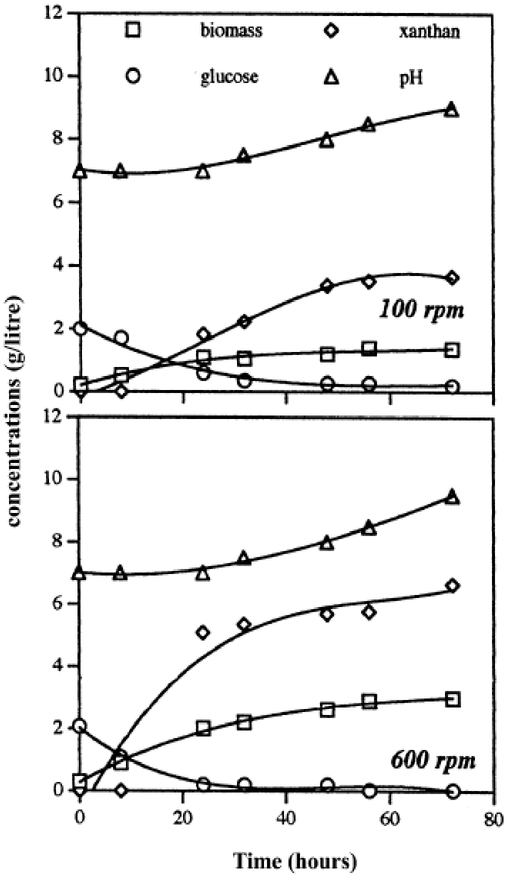


Figure 12: 72 hour Fermentation of Xanthan Gum (Xanthan Production by Xanthomonas Campestris in Batch Cultures**)**

However, an alternative method of fermentation using a centrifugal packed bed reactor (CPBR) was looked into due to the large number of fermenters required in order to produce our xanthan gum using the traditional form of fermentation. The CFPB is very ideal since it produces a cell free broth by immobilizing the sells during the fermentation. Therefore the separation process will be less rigorous. In addition, CPBRs constantly circulate their broth from the bottom of the vessel to the top, where it is spray dried in order to increase the aeration factor of the broth, which was the limitation with traditional fermentation of xanthan gum.

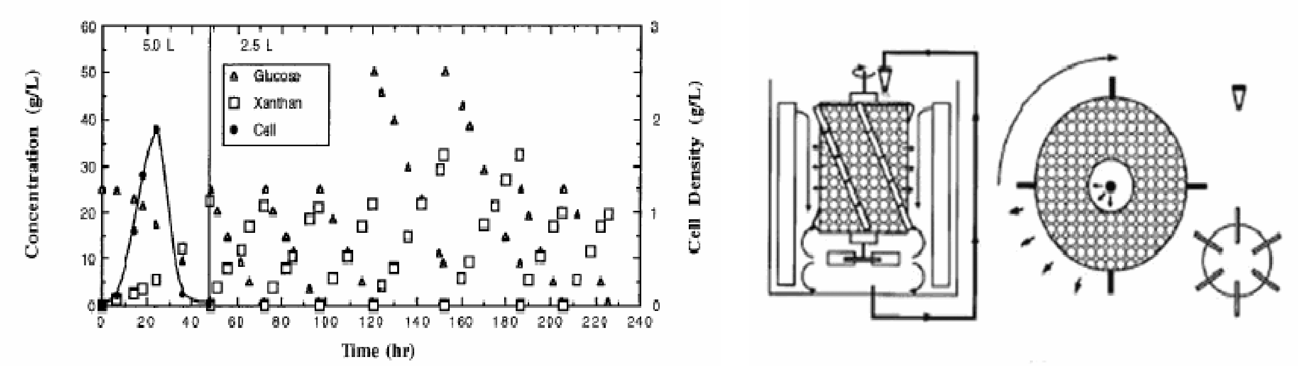


Figure 13:CPBR production of xanthan gum (left) CFPB schematic (right). (Centrifugal Fibrous Bed Reactor**)**

The figure above was used in order to determine the initial glucose concentration, the length required to ferment that particular amount of glucose, and the resulting production of xanthan. The total volume required for fermentation can be solved for with the initial glucose concentration, in this case 50 g/L, and the total amount of glucose available, i.e. 23 tons/batch for 1 mi^2 of algae with 20% lipids. Once the total volume has been determined the final amount of products in the system can then be found by multiplying the end concentration of each product by the total volume.

Inoculation Tanks

Inoculation tanks are used to grow initial cultures of microbes for each fermenter. These tanks are sized to produce 1% of the total volume of the larger fermentation tanks. Inoculation is an advantageous way of increasing biomass rapidly so that once transferred to the fermentation tanks time is not wasted producing biomass when it can be better used fermenting sugars.

Fermentation Tanks

The total volume required for fermenting the glucose produced by 1 mi^2 of algae with 20% lipids every 24 hours, or 1 batch, is about 502 m^3, whereas the volume required for fermenting the glucose of 4 mi^2 and 7 mi^2 is about 1880 m^3 and 3270 m^3. Using a standard fermentation tank 10 m tall and 4 m in diameter it would require between 3 and

26 fermentation tanks depending on the amount of algae being grown in mi^2, with each fermentation tank costing about $289,000.

The Separation Process

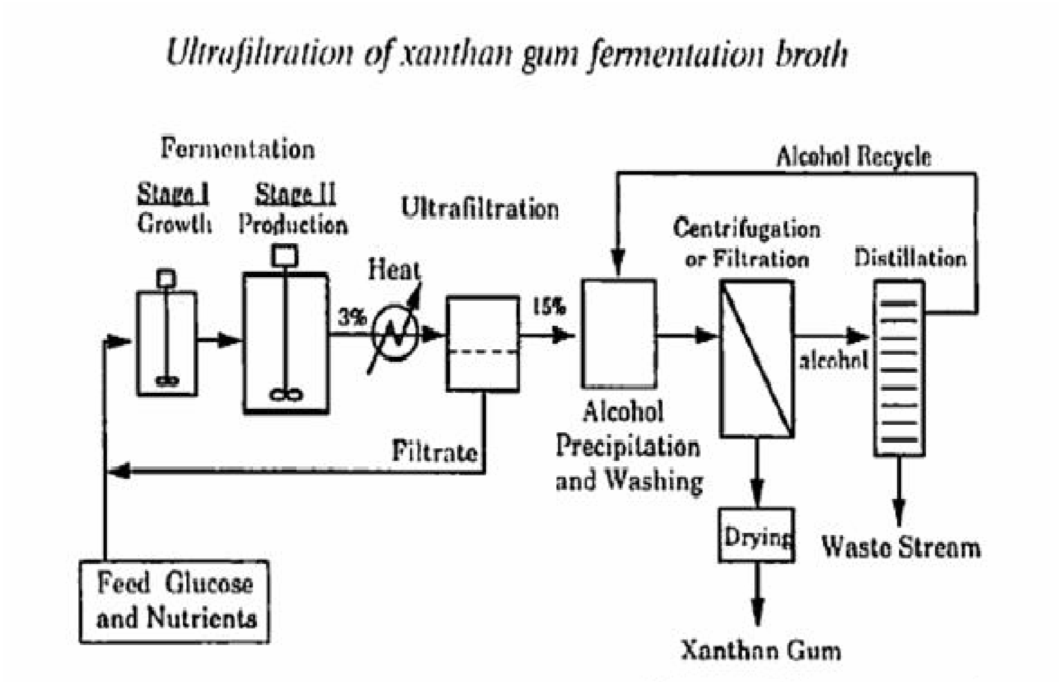


Figure 14: The separation process flow diagram for xanthan gum production (Ultrafiltration of Xanthan Gum).

The first step involved with the purified out xanthan gum is to heat treat the broth as it exits the fermentation vessel. This is done so that all microbial growth is halted and killed off. The broth is then sent through ultrafiltration mainly to reduce the xanthan rich broth size. The filtrate is then recycled back to the fermenter for further processing of any unfermented sugars. The remainder is then precipitated with 2 parts isoproanol for each part of xanthan rich broth. The precipitated xanthan gum is then filtered from the rest of the solution and sent on to be dried and milled. Finally, the isopropanol is distilled and then recycled for additional precipitations.

Protein

The protein associated with agricultural biomass is often sold as an added protein supplement for cattle and other livestock. The sugar depleted protein rich algal biomass is going to be sold to the cattle industry at a price of $200 per ton, this results in an annual net sales of 3.3 MM$ per square mile of 20% lipid algae. As far as precautions when working with the protein rich solution, it would be recommended to store the protein is a clean dry are until it is shipped out to its buyer. This is done in order to avoid spoilage of the protein in the event that it came into contact with water.

Glycerol

The transesterfication process generates approximately 101,000 gallons of glycerol per square mile per year with a lipid content of 20% in our algae. Due to a large glycerol market of 200,000 tons a year, the glycerol produced during transesterfication is going to be sold on the market at a value of $0.128 per gallon, resulting in $13,000 glycerol revenue a year. (**Glycerol**)

Algae Genetic Engineering

The possibility of genetic manipulation was studied with the understanding that genetic modifications could further increase the lipid concentration within the algal oil source. This is important since the lipid content within the algae relates directly to the amount of biodiesel that can be produced per area of algae harvested. With extreme expenses associated with the harvesting process it would be very desirable to reduce the volume of water filtered. If the algae grow to the same concentrations both before and after genetic manipulation and increasing the lipid content within the algae via genetic modifications results in a smaller amount of algae required to produce the same amount of biodiesel, the volume of water required to produce some predetermined amount of biodiesel would be reduced. For example,

Non-genetically modified lipid concentration = 20 % wt
Genetically modified lipid concentration = 40 % wt
Density of algae in the ponds after growing cycle = 140 ton / hectare

If a 1 hectare pond was used to grow the non-genetically modified algae, the resultant amount of lipid produced would be:
(20 %) (140 ton / hectare) (1 hectare) = 28 ton lipid

Now if the same amount of lipid was desired to be produced, but this time with the genetically modified algae, the resultant amount of land required would be:
(40 %) (140 ton / hectare) (x hectare) = 28 ton lipid

Solving the second equation for x, the amount of hectares required to produce 28 ton lipid, gives x = 0.5 hectare. This is a significant reduction the amount of land required to produce the desired amount of lipid; this reduced land area also results in a reduced amount of water filtration.

With the ability to genetically modify organisms there comes a grave responsibility. An ecosystem is a very delicate system of prey and predators. Should the predator gain an edge when hunting their prey, the predator could over hunt the prey resulting in diminishing numbers of prey. As the prey die off from being over hunted the predators will eventually begin to starve as their food source (the prey) become scarcer and scarcer. Thus, it should be known that the predator prey relationship is very delicate and only the greatest care should be taken when altering the genetic make up of an organism in order to avoid giving the organism an unnatural advantage that might disrupt the food chain.

In order to begin the genetic engineering process an initial study is required to determine the organism's genome, which contains all of the genetic information required to reproduce and maintain a living example of the particular organism being studied. The genetic information contained within the genome is stored in DNA and divided into subunits known as genes.

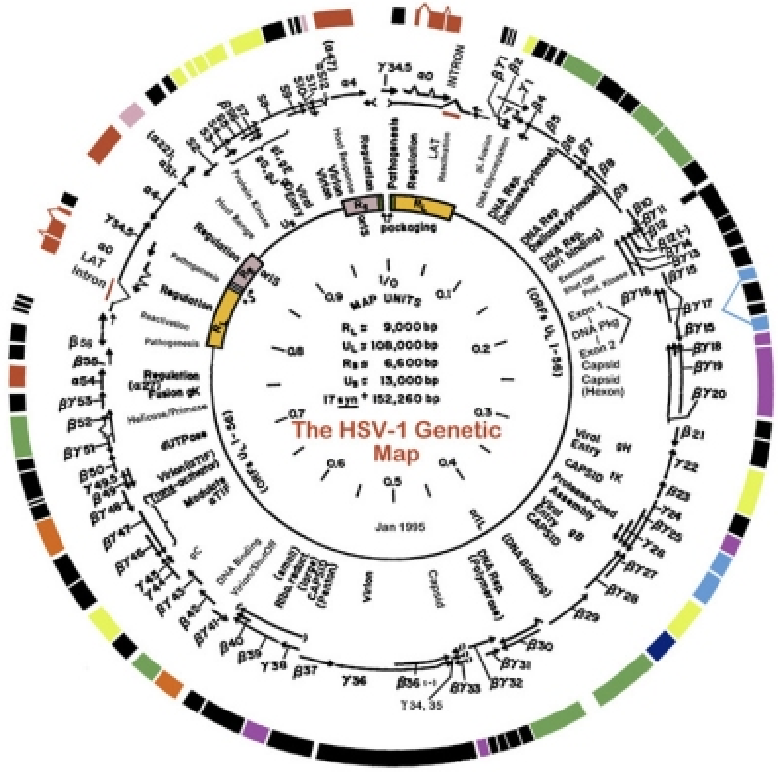


Figure 15: Herpes Simplex Virus (HSV-1) genetic map (Genome HSV-1**).**

The genetic map is an important tool since it allows for the location of genes that express certain characteristics or traits within the organism. However, there has yet to be a genome study conducted on the two particular algae that are being considered for this facility's oil source. One particularly valuable technique widely used to determine an organism's genome, when are where particular genes are expressed, is known as reverse transcription polymerase chain reaction (RT-PCR). Essentially this is PCR followed by reverse transcription, the process of converting RNA to its complementary DNA strand. PCR is a biochemistry technique that begins by denaturing the DNA double helix. Then, primers are bound to a desired location in the single stranded DNA and when placed under the proper annealing conditions, the DNA can be rapidly (exponentially) and accurately replicated. The conditions for PCR are:

1. DENATURATION at 94 ^{o}C
2. ANNEALING at 54 ^{o}C

3. REPLICATION at 72 °C
4. RINSE AND REPEAT (steps 1 through 3)(**Principle of PCR**)

The resulting gene replication process can be achieved by repeating the above conditions.

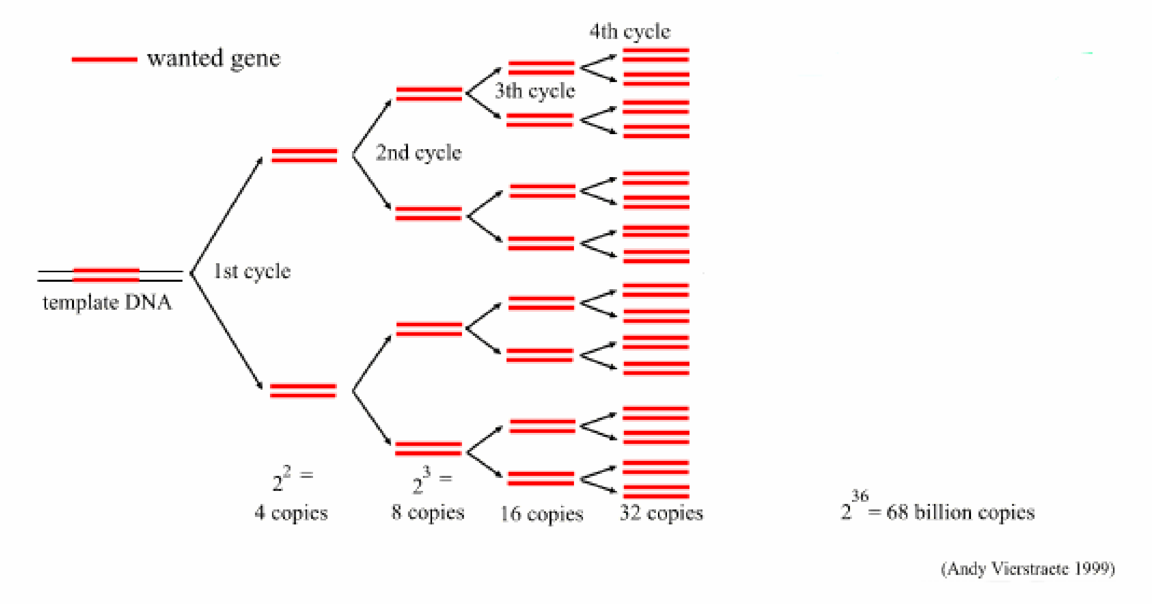


Figure 16: Gene replication with PCR (Principle of PCR**).**

The following illustrates the steps required to produce a complementary DNA strand from RNA.

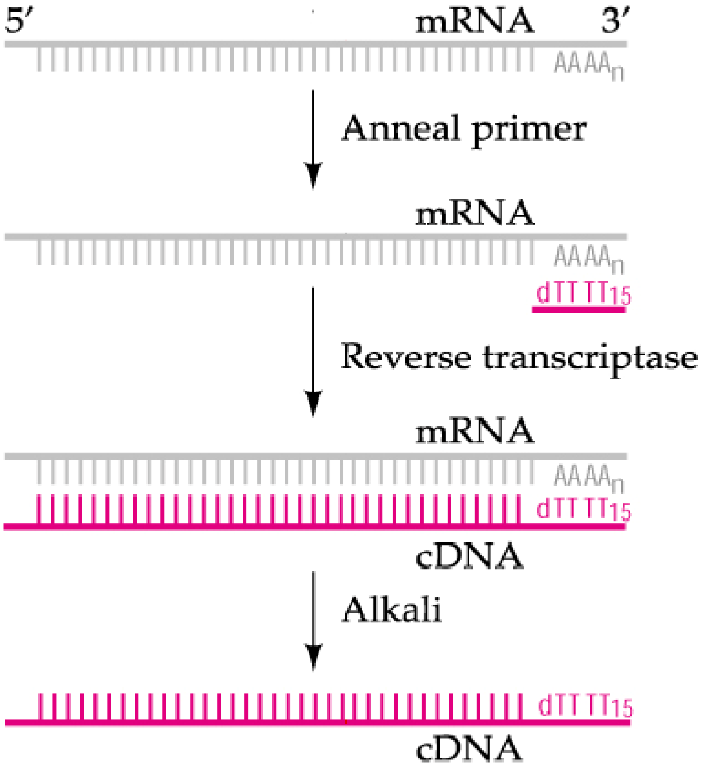


Figure 17: Synthesis of complementary DNA from RNA(R reverse transcription**).**

The advantage of producing the complementary DNA (cDNA) stand is that it can be separated from the solution quite readily. Once reverse transcription has taken place, thus the mRNA cDNA double helix has been completed, it can be uncoiled by raising the pH. Next, enzymes can be added that target the mRNA and cleave it into smaller subunits. From there the mRNA and cDNA can be separated based on molecular weight. This is possible since the mRNA was cleaved enzymatically and should have a substantially smaller molecular weight compared to the cDNA, which remains in its full chain length.

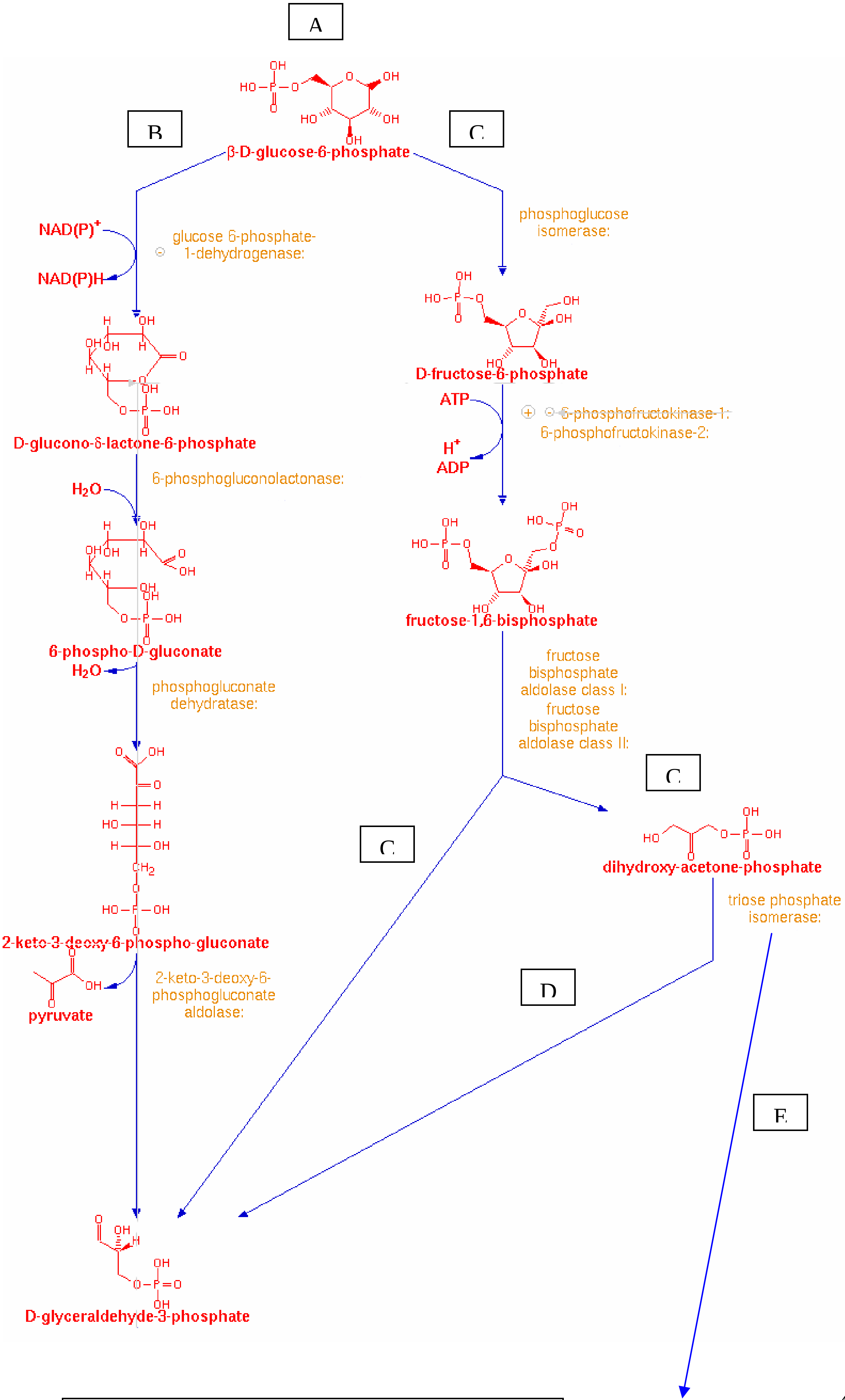
After the genetic maps of the particular algae under consideration have been identified, the genetic engineering can be conduction, however without an actual genome the genetic engineering will be continued on a theoretical basis from here on out. With that said, there are three main uses of genetic engineering: loss of function, gain of function, and tracking. Loss of function processes are used when an organism has an undesirable trait. The trait is related to a particular gene within the genetic code and then it is removed through a process known as gene knocking. Next there are gain of function processes, which are opposite of loss of function. Instead of removing traits, there is a particular trait that is desired within the organism and therefore the desired trait is added to the organism's genetic code by a process known as gene splicing. Finally, genetic material can be modified to assist in tracking particular traits, for example florescent green jellyfish protein is often added to organisms so that certain genes will fluoresce under ultraviolet light.

Looking back to the purpose of the genetic modifications, it was desired to increase the lipid content within our select algae. Thus the lipid content is desired to increase by removing certain metabolic pathways. Thus, this may initially be viewed as loss of function process. However, due to a lack of information on our particular organism it was concluded that removing genes could have a devastating effect upon the lifecycle of the organism, primarily because it's not understood how each reaction affects the overall lifecycle. Therefore, rather than remove the gene totally and risk ruining our specimen, an inhibitor is going to be spliced into the algae's genetic information that slows the undesirable pathway rather than terminating it altogether.

In order to determine the effects of the genetic modification on a theoretical basis an analysis had to be conducted on the metabolic processes leading up to triglycerol biosynthesis. Looking into the algal energy source, photosynthesis, it was found that glucose was a product of the photosynthesis reaction.

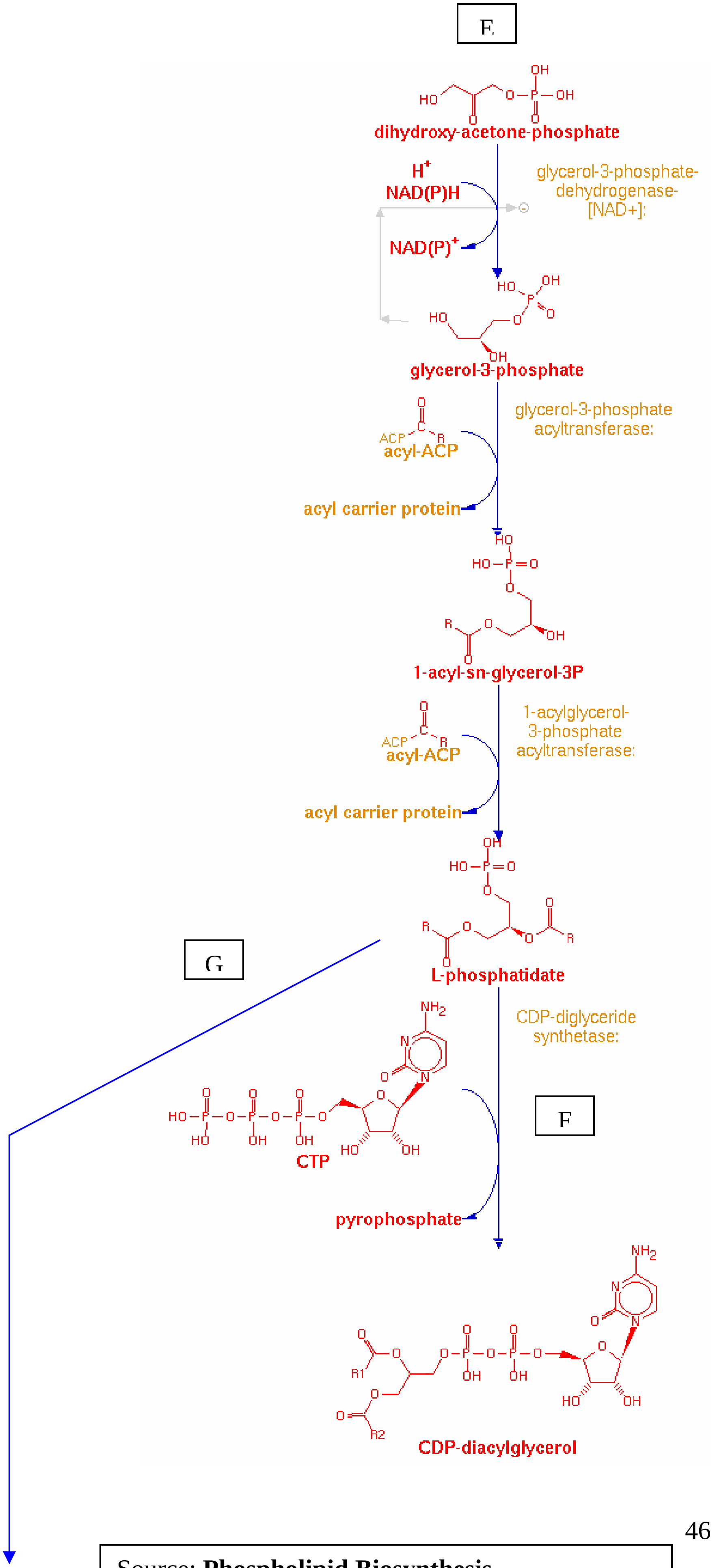
$$6H_2O + 6CO_2 \rightarrow C_6H_{12}O_6 + 6O_2$$

This glucose is then converted to glucose-6-phosphate (G-6-P) through a series of reactions in which ATP is broken down into ADP. The resulting G-6-P is then converted through a series of reactions into pyruvate which can then be used as a source of energy production within the cell. This provided a starting point for the triglycerol synthesis. From there all the reactions were cascaded in order to determine which reactions could be inhibited in order to push the remained of reactions in a desired direction.



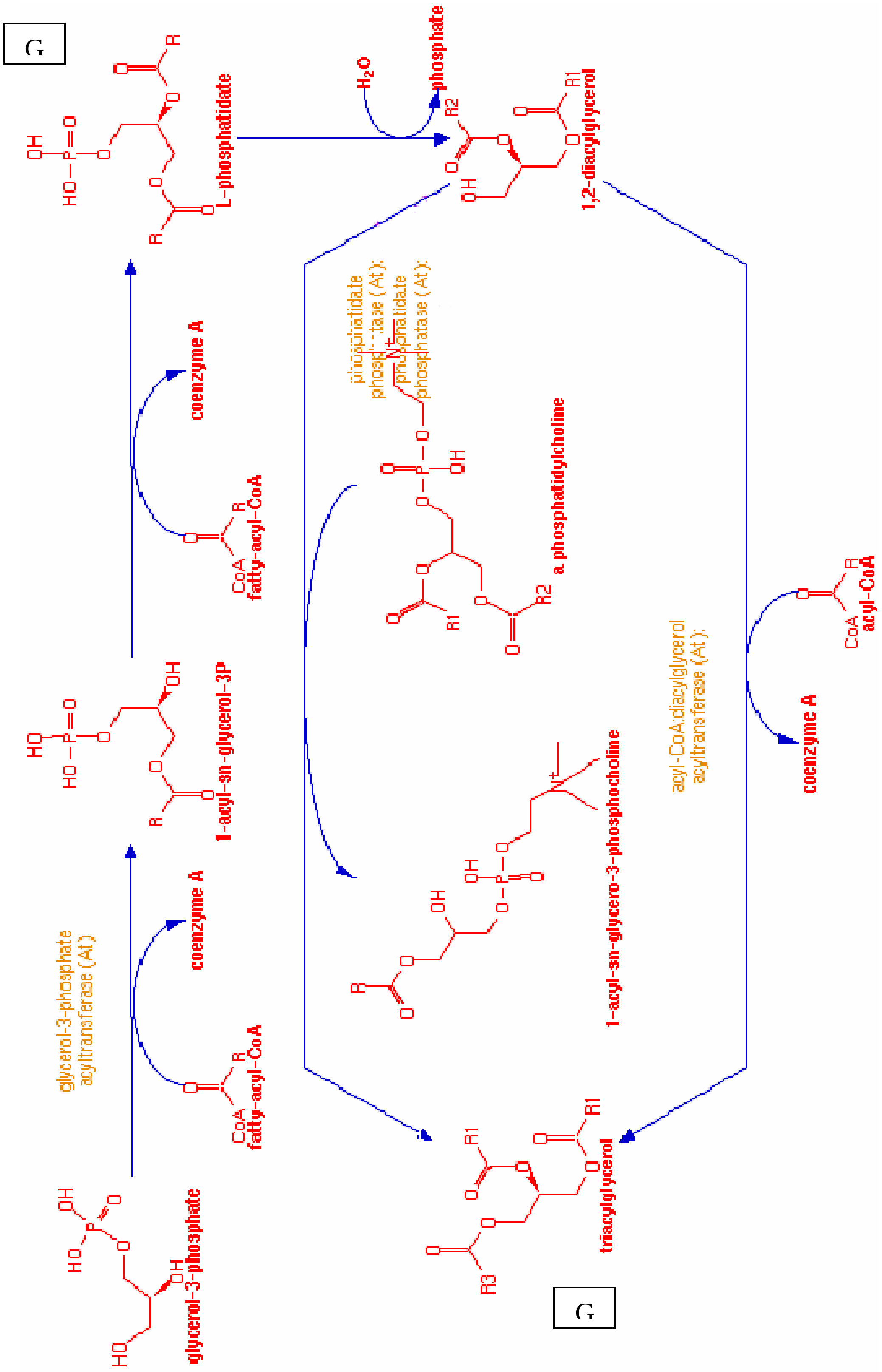
Source: **Entner Dourvoroff & Glycolysis**

Figure 18: Entner Dourvoroff & Glycolysis



Source: **Phospholipid Biosynthesis**

Figure 19: Phospholipid Biosynthesis



Source: **Triglycerol Biosynthesis**
Figure 20: Triglycerol Biosynthesis

Since the genome of the algae was unknown it was assumed that competing reactions proceeded equal in both directions, for example if one had 10 mol A and a complete reaction was assumed, with X → Y and X → Z, theoretically one would end up with 5 mol B and 5 mol C.

An analysis of the above reactions determined the following competing reactions:

A → B and A → C C → D and C → E E → F and E → G

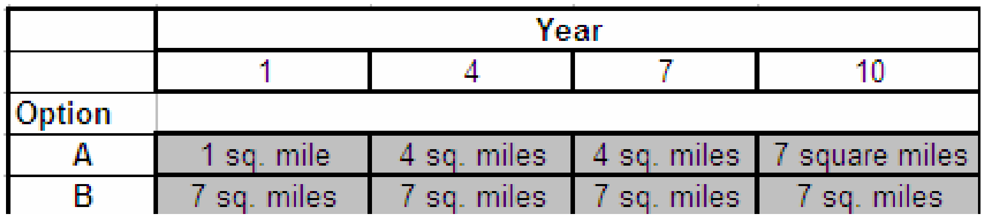
The initial study was conducted with the understanding that blue-green algae are cyanobacteria. Bacteria make use of the Entner-Doudoroff pathway as a means of degrading G-6-P, however it was later determined that our organisms would not process through this pathways and therefore would degrade the G-6-P through the glycolysis degradation pathway. Assuming a 100 mol basis of G-6-P, the original pathway produced 25 mol triglyceride per 100 mol G-6-P. If the competitive reactions that were initially assumed to proceed equally in each direction were inhibited such that the reaction that cascaded to triglyceride was promoted 56.25 mol triglyceride were produced per 100 mol G-6-P

In conclusion, with little available information on the algae being considered further analysis could not be conducted. Provided that the genome was available one could look up the enzymes responsible for accelerating each reaction. Once the enzyme is determined it is possible to associate the enzyme with a particular inhibitor and possibly its genetic information that would have to be spliced into the algal DNA.

Economic Analysis

The net present worth (NPW) of two different design options was evaluated along with the return on investment (ROI) to compare their profitability. A reminder of these options is in Table 7 below.

Table 7: Design Options



	Year			
	1	4	7	10
Option				
A	1 sq. mile	4 sq. miles	4 sq. miles	7 square miles
B	7 sq. miles	7 sq. miles	7 sq. miles	7 sq. miles

Option A is an expansion that was chosen to compare to Option B which started at maximum land utilization. The reason for comparing these particular options is to see if starting out with a lower fixed capital investment (FCI) might yield a profit sooner and allow for reinvestment of that profit. The FCI, TCI, NPW and ROI of both option A and B are in Table 8 below.

Table 8: NPW at $0.72/gallon

Option	Lipid Content	TCI	FCI	NPW	ROI
A	20%	574,000,000	488,000,000	-356,000,000	-62%
	30%	569,000,000	484,000,000	-407,000,000	-71%
	40%	563,000,000	479,000,000	-440,000,000	-78%
B	20%	364,000,000	309,000,000	13,000,000	3%
	30%	362,000,000	308,000,000	-104,000,000	-29%
	40%	359,000,000	306,000,000	-177,000,000	-49%

Option A is not profitable at the determined selling price of $0.72/gallon and Option B is only profitable for the 20% lipid production with a very low ROI of 3%. This is due to the incredibly large TCI range of $300 to ~$600 millon.

The selling price of biodiesel was varied from the chosen biodiesel selling price of $0.72/gallon to determine when both options were profitable. For option A to be profitable, the selling price would have to be $9.5/gallon. This is far too high to be realistic, so option A would not be recommended. A risk analysis was performed by varying the selling price of biodiesel by 50% because of the $9.5/gallon price required for profitability of option A. The following risk curves encompass the 20%, 30%, and 40% lipid content scenarios.

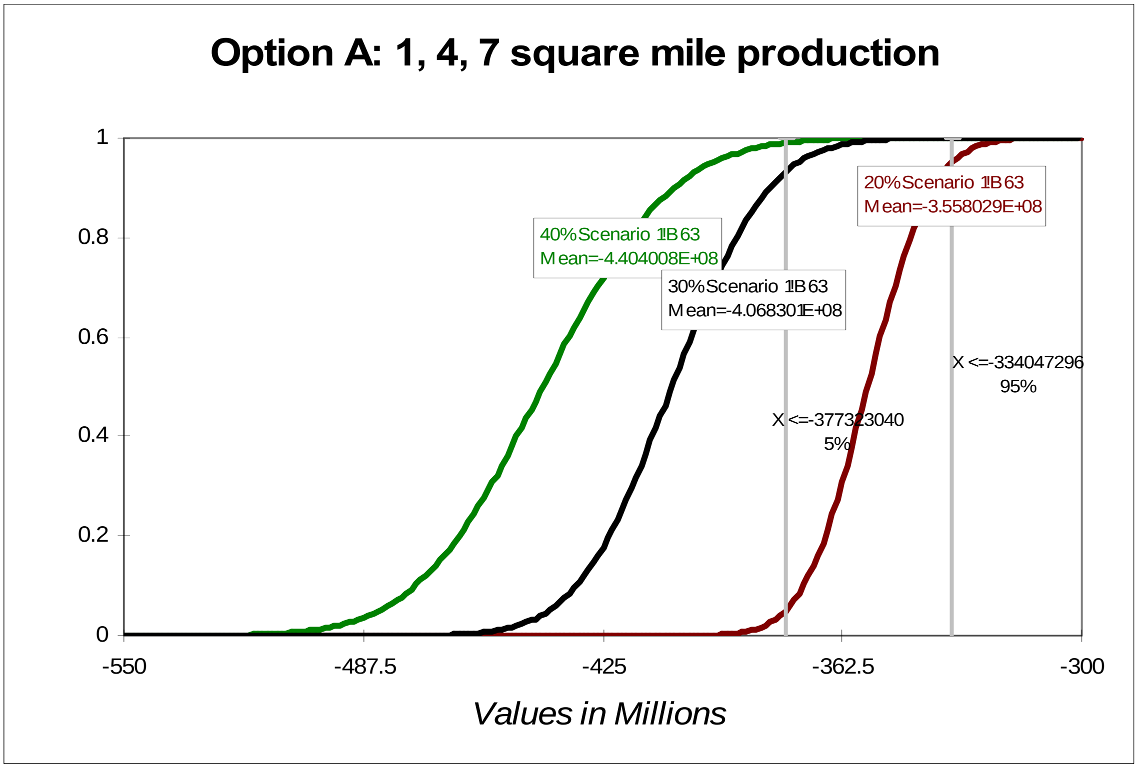


Figure 21: Option A Risk

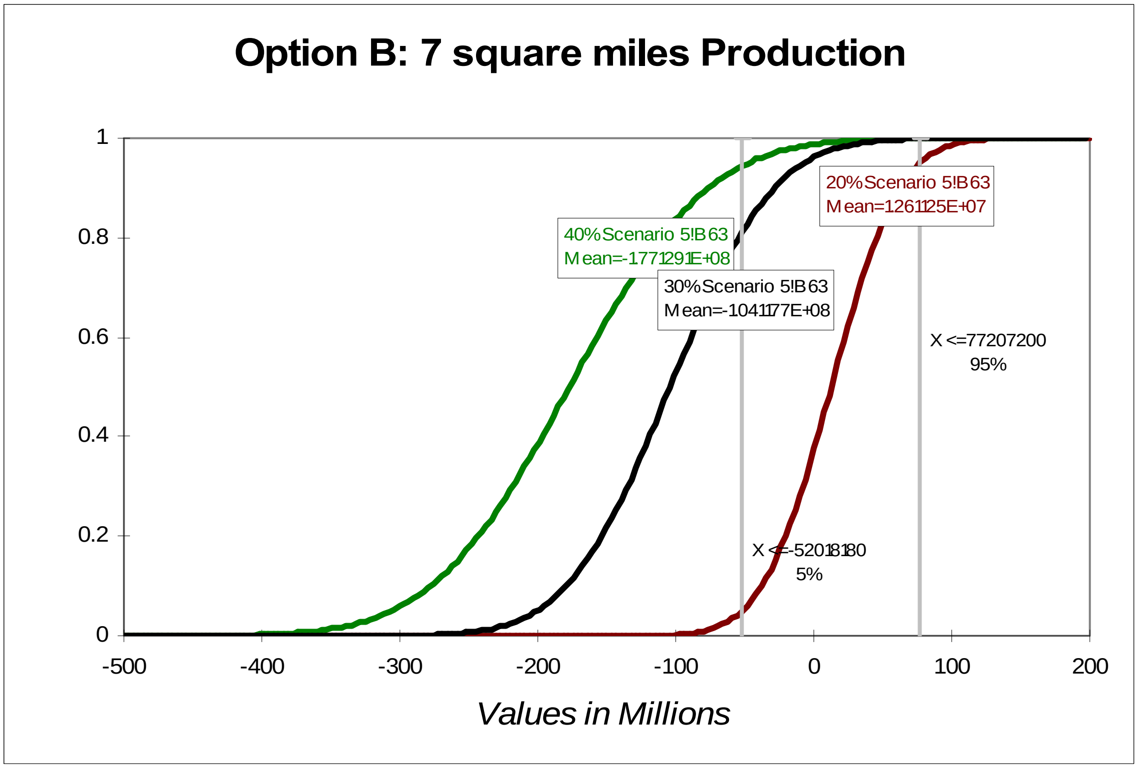


Figure 22: Option B Risk

As seen in the Figure 21 and 22 above , the risk for the 20% curve is the least. There is a trend that shows as the lipid content increases the probability of making money decreases. This is because the byproduct from fermentation, xanthan gum, is more profitable than the biodiesel. With lower lipid content in the algae, there are more sugars and therefore more xanthan gum that can be produced. In option A, there is no chance of making money as we stated previously. For option B, however, varying the price 50% makes the 30% and 40% lipid content have a small probability of making money. The 20% lipid in option B, is by far the most promising, but it still has a 40% chance of losing money.

In order to see when biodiesel becomes more profitable than xanthan gum, the selling price was again varied. Table 9 below shows the results.

Table 9: Break Even Price for Biodiesel over Xanthan Gum

		Net Present Worth (15 years)			
	Selling Price of Biodiesel	$0.72	$1.50	$2	$3.14
Option A	20% Lipid	-$356,000,000	-$324,000,000	-$304,000,000	-$257,220,000
	30% Lipid	-$407,000,000	-$359,000,000	-$329,000,000	-$258,950,000
	40% Lipid	-$440,000,000	-$377,000,000	-$336,000,000	-$243,200,000
Option B	20% Lipid	$13,000,000	74,000,000	$113,000,000	$202,800,000
	30% Lipid	-$104,000,000	-12,000,000	$47,000,000	$181,100,000
	40% Lipid	-$177,000,000	-55,000,000	$24,000,000	$203,200,000

At a selling price of $3.14/gallon, the biodiesel just surpasses the xanthan gum in profitability. It is possible for the selling price to be increased to this amount, however, that would require selling the pure biodiesel directly to a diesel gas station at the same price for regular diesel or a little more. At this selling price the ROI is around 50% for option B for the different lipid content. This is exhibited in Table X below.

Table 10: ROI

Option	Lipid Content	TCI	FCI	NPW	ROI
A	20%	574,000,000	488,000,000	-257,000,000	-45%
	30%	569,000,000	484,000,000	-259,000,000	-45%
	40%	563,000,000	479,000,000	-243,000,000	-43%
B	20%	364,000,000	309,000,000	203,000,000	56%
	30%	362,000,000	308,000,000	181,000,000	50%

	40%	359,000,000	306,000,000	203,000,000	57%

Increasing the revenue by increasing the selling price compensates for the extremely high capital investment partially. If the TCI can be reduced, the profitability of biodiesel from algae could greatly increase. The risk would also decrease with lower FCI. A cost break down of the major different processes equipment cost is in Figure 23 below.

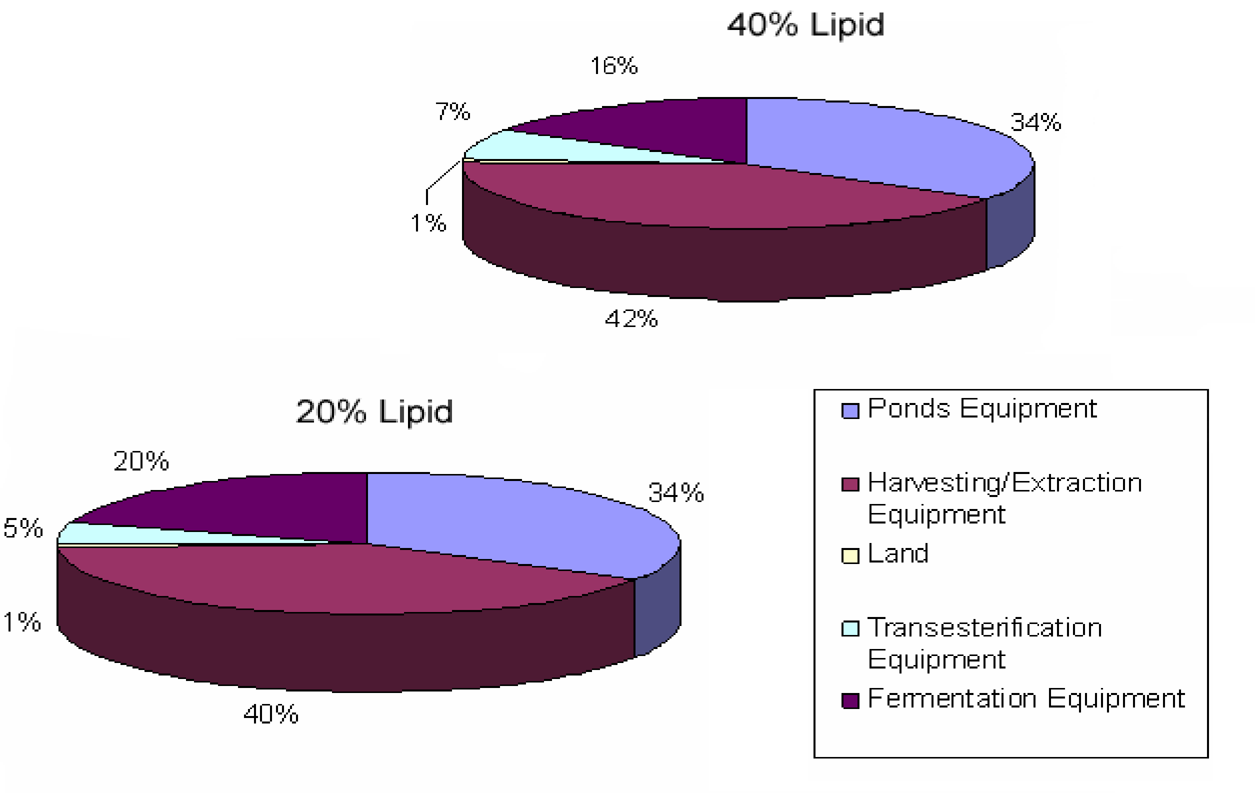


Figure 23: Cost Breakdown

It can be seen that the major costs are harvesting and oil extraction. It also shows that with increasing lipid content your transesterfication equipment costs increases, while the fermentation cost decreases. This coincides with what was stated before: lower lipid content means higher sugar content. The more sugar that is available, the more equipment that is required to ferment it. The less lipids that are available the less amount of money that will have to be spent converting them to biodiesel.

In conclusion, option B is more profitable and therefore the better choice. However, it still is very risk and there is a reasonable 0.4 probability of losing a great amount of money. If the cost of harvesting the algae and extracting the oil can be reduced by optimizing the process or finding better cheaper ways to dewater the algae and extract the oil, then the TCI will decrease along with the risk.

Another significant cost is the pond excavation/building cost. There is room for optimization to reduce these costs, too. With more extensive research into these aspects of the algae biodiesel process, cost can be reduced.

Conclusions and Recommendations

For this newly developing technology, there are still many questions unanswered and with them comes a high level of uncertainty and risk. Algae shows good potential as a source for biodiesel with high yields of lipid and minimal land usage in comparison with agricultural crops. For the two options explored, the expansion process was not profitable at the determined selling price of \$0.72/gallon biodiesel. The 7 square mile option was profitable at 20% lipid at this selling price. There is a high cost of harvesting and extracting oil from algae. Very high TCI makes the ROI very low for 15 years at the selling price of \$0.72/gallon. With the source of algae, the base catalyzed transesterification reaction is sufficient to produce a high yield of biodiesel. With the throughput of algae and biomass that this facility processes the optimum choice for byproducts is fermentation, specifically fermentation to yield xanthan gum. At the current chosen selling price, xanthum gum is more profitable than biodiesel. A significant increase in price will yield a higher profitability of biodiesel over xanthan gum. Other scenarios of production can be explored to see their profitability. In the economics of this process, tax incentives were not utilized, so in future analysis they should be incorporated.

Future Research and Plans

In the future, a more thorough search on the types of algae in the El Paso area would minimize the major uncertainty of biodiesel production from algae. It would be preferable if samples from the exact area could be taken and a general consensus be concluded on the dominant species of the region. Whether or not the species is high in lipid content, it is most desirable for it to be adaptable to a varying salinity environment. Genetic engineering can be implemented in order to maximize the lipid content, once survival of the species in the environment is ensured. One of the major costs of concern is associated with harvesting of the algae. Algae has very small particles and this becomes very costly to separate from water. Additionally the amount of energy required to extract the oil from the algae is extensive. Different ways to minimize the cost of these processes should be explored to maximize profit. As diesel prices continue to increase, research into the economical feasibility of bioreactors should be explored because of their ability to capture flue gas a maximize its utilization in the production of algae. Bioreactors also give more controlled environments and this would help to lower risk.

References

Becker, E W. Microalgae: Biotechnology and Microbiology. New York: Cambridge UP, 1994. 80-180.

Bridgewater, A. Fast Pyrolysis of Biomass: a Handbook. Newbury, UK: Cpl P, 1999.

Leung, Wallace W. Industrial Centrifugation Technology. New York: McGraw-Hill, 1998.

Peters, Max S., Klaus D. Timmerhaus, and Ronald E. West. Plant Design and Economics for Chemical Engineers. 5th ed. Madison, Wisconsin: McGraw Hill, 2004.

Reisman, Harold B. Economic Analysis of Fermentation Processes. Boca Raton, FL: CRC P, 1988.

Stevens, Christian, and Roland Verhe. Renewable Bioresources: Scope and Modification for Non-Food Applications. West Sussex, England: John Wiley & Sons, Ltd, 2004.

Ward, Owen P., and Englewood Cliffs. Fermentation Biotechnology. New Jersey: Prentice Hall, 1989.

*** additional references can be found in the supporting material folder in pdf format, pdf citations are in parenthesis in bold